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Molecular Diagnostic Testing by eyeGENE: Analysis of Patients With Hereditary Retinal Dystrophy Phenotypes Involving Central Vision Loss

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Purpose. To analyze the genetic test results of probands referred to eyeGENE with a diagnosis of hereditary maculopathy.

Methods. Patients with Best macular dystrophy (BMD), Doyne honeycomb retinal dystrophy (DHRD), Sorsby fundus dystrophy (SFD), or late-onset retinal degeneration (LORD) were screened for mutations in *BEST1*, *EFEMP1*, *TIMP3*, and *CTRP5*, respectively. Patients with pattern dystrophy (PD) were screened for mutations in *PRPH2*, *BEST1*, *ELOVL4*, *CTRP5*, and *ABCA4*; patients with cone-rod dystrophy (CRD) were screened for mutations in *CRX*, *ABCA4*, *PRPH2*, *ELOVL4*, and the c.2513G>A p.Arg838His variant in *GUCY2D*. Mutation analysis was performed by dideoxy sequencing. Impact of novel variants was evaluated using the computational tool PolyPhen.

Results. Among the 213 unrelated patients, 38 had BMD, 26 DHRD, 74 PD, 8 SFD, 6 LORD, and 54 CRD; six had both PD and BMD, and one had no specific clinical diagnosis. *BEST1* variants were identified in 25 BMD patients, five with novel variants of unknown significance (VUS). Among the five patients with VUS, one was diagnosed with both BMD and PD. A novel *EFEMP1* variant was identified in one DHRD patient. *TIMP3* novel variants were found in two SFD patients, *PRPH2* variants in 14 PD patients, *ABCA4* variants in four PD patients, and p.Arg838His *GUCY2D* mutation in six patients diagnosed with dominant CRD; one patient additionally had a *CRX* VUS. *ABCA4* mutations were identified in 15 patients with recessive CRD.

Conclusions. Of the 213 samples, 55 patients (26%) had known causative mutations, and 13 (6%) patients had a VUS that was possibly pathogenic. Overall, selective screening for mutations in *BEST1*, *PRPH2*, and *ABCA4* would likely yield the highest success rate in identifying the genetic basis for macular dystrophy phenotypes. Because of the overlap in phenotypes between BMD and PD, it would be beneficial to screen genes associated with both diseases.

Keywords: genetic testing, eyeGENE, macular dystrophy

The National Ophthalmic Disease Genotyping and Phenotyping Network, eyeGENE, is a multicomponent genetics initiative created by the National Eye Institute to facilitate research in inherited eye disease. The program provides access to deidentified DNA, phenotype, and genotype information of its participants.[1] This study describes eyeGENE data on six specific conditions involving central vision loss: Best macular dystrophy, Doyne honeycomb retinal dystrophy, Sorsby fundus dystrophy, late-onset retinal degeneration, pattern dystrophy, and dominant and recessive cone-rod dystrophy. All of these conditions are autosomal dominant inherited diseases except recessive cone-rod dystrophy (CRD).

Best macular dystrophy (BMD) is characterized by a yellow yolk-like lesion in the macula with loss of central vision. Onset of disease is generally in childhood or early teenage years. Mutations in the bestrophin-1 (*BEST1* or *VMD2*) gene are

known to cause BMD[2,3] (Table 1). Although BMD is an autosomal dominant disease, recessive mutations in *BEST1* are associated with recessive bestrophinopathy with symptoms similar to those of dominant BMD.[4]

Doyne honeycomb retinal dystrophy (DHRD) or malattia leventinese is clinically diagnosed by the presence of drusen in the (sub)retinal epithelium (RPE) of the posterior region of the eye, especially in areas associated with the macula and the optic disc. Over time, a honeycomb pattern of drusen deposition can be seen in the fundus of some patients. Mutations in the epidermal growth factor containing fibulin-like extracellular matrix protein 1 gene (*EFEMP1*) are associated with DHRD[5] (Table 1).

Sorsby fundus dystrophy (SFD) is a late-onset disease with central vision loss but also includes atrophy of the peripheral choroid, drusen-like deposits, and choroidal neovascularization

TABLE 1. Retinal Disease Genes Screened for Mutations and the Phenotypes Associated With These Genes

Genes Screened	Location	Gene Accession Number	Chromosome	Associated Diagnosis
ABCA4	1p22.1	NM_000350.2	1	Pattern dystrophy, cone-rod dystrophy
				Recessive retinitis pigmentosa
BEST1	11q12.3	NM_004183.3	11	Best macular dystrophy
CRX	19q13.32		19	Dominant cone-rod dystrophy
CTRP5	11q23.3	NM_001278431.1	11	Late-onset retinal degeneration
EFEMP1	2p16.1	NM_001039348.2	2	Doyne honeycomb retinal dystrophy
ELOVL4	6q14.1	NM_022726.3	6	Autosomal dominant Stargardt's-like macular dystrophy
GUCY2D	17p13.1	NM_000180.3	17	Dominant cone-rod dystrophy
PRPH2	6p21.1	NM_000322.4	6	Pattern dystrophy, cone-rod dystrophy
				Macular degeneration
TIMP3	22q12.3	NM_000362.4	22	Sorsby fundus dystrophy

in most cases.[6] Mutations in the tissue inhibitor of metallopeptidase 3 gene (*TIMP3*) have been implicated in the development of SFD[7] (Table 1). Due to the late age of onset, overlap in clinical symptoms, and lack of information on additional family members, SFD is often misdiagnosed as the more common condition, age-related macular degeneration (AMD).

Late-onset retinal degeneration (LORD) is a disease with early onset of abnormal lens zonules, abnormalities in dark adaptation, late-onset drusen-like deposits, central vision loss, and neovascularization of the retina.[8] A single missense mutation, p.Ser163Arg, in the C1q tumor necrosis factor-related protein 5 gene (*CqQTNF5/CTRP5*) has been reported in patients with LORD[9] (Table 1). The clinical symptoms of LORD overlap with the symptoms of SFD and AMD.

Pattern dystrophy (PD) patients often exhibit yellow, orange, or gray deposits in the macula that disrupt central vision. Occasionally, the pattern will appear in the shape of a butterfly.[10] Mutations in the peripherin 2 (*PRPH2*) and adenosine triphosphate (ATP) binding cassette subfamily A, member 4 (*ABCA4*) genes have been reported in patients with the PD phenotype[10] (Table 1).

Cone-rod dystrophies are characterized by peripheral vision loss along with central vision loss, and this phenotype is inherited in both dominant and recessive fashion. Dominant CRD is caused by mutations in the guanylate cyclase 2D (*GUCY2D*) and the cone-rod homeobox (*CRX*) genes[11–13] (Table 1). Mutations changing the codon arginine at amino acid position 838 to histidine, proline, or cysteine in *GUCY2D* have been identified in unrelated patients with CRD. The p.Arg828His mutation is the most common among all *GUCY2D* mutations.[13] Recessive CRD is usually associated with missense mutations in *ABCA4*.[14]

We summarize the genetic variations identified in 213 patients from the eyeGENE database with a retinal dystrophy phenotype involving central vision loss.

METHODS

Since genetic and phenotypic heterogeneity are common in retinal degenerations with central vision loss, analysis of multiple genes may be needed to determine the underlying cause of these diseases. Molecular analysis by eyeGENE involves systematic screening of relevant genes beginning with the most likely to be associated with the patient's clinical phenotype as available through collaborating CLIA (Clinical Laboratory Improvement Amendment) laboratories. All research followed the tenets of the Declaration of Helsinki and had Institutional Review Board approval.

Two hundred thirteen patients listed in the eyeGENE database with a retinal dystrophy phenotype of central vision loss were selected for this study. Mutation screening was carried out by arrayed primer extension (APEX) technology and/or dideoxy sequencing by eyeGENE collaborating laboratories including ours (Downs CA, et al. *IOVS* 2004;45:E-Abstract 2474). The genes selected for analysis were based on the primary and secondary diagnosis provided by the physician. When mutations were not detected in genes associated with the primary diagnosis, other genes associated with similar phenotypes were tested. Patients with a primary diagnosis of Stargardt's were not included in the study.

Late-Onset Retinal Dystrophy

Six probands with a primary diagnosis of LORD were screened for mutations in exons 1 to 3 of the *CTRP5* gene.

Sorsby Fundus Dystrophy

Eight probands with a primary diagnosis for SFD were screened for mutations in exons 1 to 5 of the *TIMP3* gene.

Doyne Honeycomb Retinal Dystrophy

Twenty-six probands with a primary diagnosis for DHRD were screened for mutations in the *EFEMP1* gene (exons 1–11 in 25 patients; exons 3–12 in one patient).

Best Macular Dystrophy

Forty-four probands with a clinical diagnosis of BMD were screened for mutations in the *BEST1* gene (exons 2–11 in 40 families; exons 1–11 in 5 families).

Pattern Dystrophy

Eighty probands with a clinical diagnosis for PD were screened for mutations in the *PRPH2* gene.

Twenty-seven probands also had clinical signs consistent with Stargardt's disease (STGD1), BMD, SFD, DHRD, or other retinal dystrophies. They were additionally screened for mutations in the *ABCA4*, *ELOVL4*, *CTRP5*, *BEST1*, and/or *PRPH2* genes.

Cone-Rod Dystrophy

Fifty-four probands with a primary diagnosis of CRD were tested based on the pattern of inheritance determined by family history and/or differential diagnoses. Thirteen CRD probands with presumably autosomal dominant inheritance

TABLE 2. Mutations and Unknown Variants Detected in Patients With Central Vision Loss

Patient	Gene	Exon	DNA Change	Protein Change	Genotype	Result	PolyPhen Description	PolyPhen Score	Molecular Diagnosis
Late-onset retinal degeneration									
NA	*CTRP5*	NA	NA	NA	NA	NA			NA
Sorsby fundus dystrophy									
Patient 1	*TIMP3*	1	c.113C>G	p.Ser38Cys	Het	vAR/us	Probably damaging	1	Positive
Patient 2	*TIMP3*	1	c.113C>G	p.Ser38Cys	Het	vAR/us	Probably damaging	1	Positive
Patient 3	*TIMP3*	5	c.610A>T	p.Ser204Cys	Het	Mut			Positive
Doyne honeycomb dystrophy									
Patient 1	*EFEMP1*	9	c.1033C>T	p.Arg345Trp	Het	Mut			Positive
Patient 2	*EFEMP1*	9	c.1033C>T	p.Arg345Trp	Het	Mut			Positive
Patient 3	*EFEMP1*	IVS10	c.IVS10-14C>T	None	Het	vAR/us	NA	NA	Unconfirmed
Best macular dystrophy									
Patient 1	*BEST1*	2	c.28G>A	p.Ala10Thr	Het	Mut			Positive
Patient 2	*BEST1*	2	c.47C>T	p.Ser16Phe	Het	Mut			Positive
Patient 3	*BEST1*	2	c.72G>T	p.Trp24Cys	Het	Mut			Positive
Patient 4	*BEST1*	3	c.240C>A	p.Phe80Leu	Het	Mut			Positive
Patient 5	*BEST1*	3	c.240C>A	p.Phe80Leu	Het	Mut			Positive
Patient 6	*BEST1*	4	c.248G>C	p.Gly83Ala	Het	vAR/us	Probably damaging	1	Positive
Patient 7	*BEST1*	4	c.277T>C	p.Trp93Arg	Het	vAR/us	Probably damaging	1	Positive
Patient 8	*BEST1*	4	c.279G>C	p.Trp93Cys	Het	Mut			Positive
Patient 9	*BEST1*	6	c.652C>T	p.Arg218Cys	Het	Mut			Positive
Patient 10	*BEST1*	6	c.652C>T	p.Arg218Cys	Het	Mut			Positive
Patient 11	*BEST1*	6	c.680A>G	p.Tyr227Cys	Het	Mut			Positive
Patient 12	*BEST1*	6	c.741G>A	p.Arg218His	Het	Mut			Positive
Patient 13	*BEST1*	6	c.741G>A	p.Arg218His	Het	Mut			Positive
Patient 14	*BEST1*	7	c.727G>A	p.Ala243Thr	Het	Mut			Positive
Patient 15	*BEST1*	7	c.727G>A	p.Ala243Thr	Het	Mut			Positive
Patient 16	*BEST1*	7	c.728C>T	p.Ala243Val	Het	Mut			Positive
Patient 17	*BEST1*	7	c.728C>T	p.Ala243Val	Het	Mut			Positive
Patient 18	*BEST1*	8	c.880C>T	p.Leu294Phe	Het	vAR/us	Probably damaging	1	Positive
Patient 19	*BEST1*	8	c.887A>G	p.Asn296Ser	Het	Mut			Positive
Patient 20	*BEST1*	8	c.903T>G	p.Asp301Glu	Het	Mut			Positive
Patient 21	*BEST1*	8	c.903T>G	p.Asp301Glu	Het	Mut			Positive
Patient 22	*BEST1*	8	c.910G>A	p.Asp304Asn	Het	Mut			Positive
Patient 23	*BEST1*	8	c.925T>C	p.Trp309Arg	Het	vAR/us	Probably damaging	1	Positive
Patient 24	*BEST1*	8	c.929T>C	p.Ile310Thr	Het	Mut			Positive
Patient 25, case 3	*BEST1*	4	c.250T>G	p.Phe84Val	Het	vAR/us	Probably damaging	1	Positive
Pattern dystrophy									
Patient 1	*ABCA4*	6	c.634C>T	p.Arg212Cys	Het	Mut			Positive
	ABCA4	30	c.4469G>A	p.Cys1490Tyr	Het	Mut			
Patient 2	*ABCA4*	17	c.2588G>C	p.Gly863Ala	Het	Mut			Unconfirmed
Patient 3	*ABCA4*	IVS26	c.3862+3A>G	Abnormal splicing	Het	vAR/us			Unconfirmed
Patient 4	*PRPH2*	1	c.271T>A	p.Tyr91Asn	Het	vAR/us	Probably damaging	0.909	Positive
	PRPH2	1	c.310-313del(AT)	p.Ile104Val	Het	Mut			
Patient 5, case 6	*PRPH2*	1	c.422A>G	p.Tyr141Cys	Het	Mut			Positive
Patient 6	*PRPH2*	1	c.422A>G	p.Tyr141Cys	Het	Mut			Positive
Patient 7	*PRPH2*	1	c.515G>A	p.Arg172Gln	Het	Mut			Positive
Patient 8	*PRPH2*	2	c.583C>T	p.Arg195Stop	Het	Mut			Positive
Patient 9	*PRPH2*	2	c.629C>G	p.Pro210Arg	Het	Mut			Positive
Patient 10	*PRPH2*	2	c.635G>C	p.Ser212Thr	Het	Mut			Positive
Patient 11	*PRPH2*	2	c.683C>T	p.Thr228Ile	Het	Mut			Positive
Patient 12	*PRPH2*	2	c.708C>G	p.Tyr236Stop	Het	Mut			Positive
Patient 13, case 4	*PRPH2*	IVS2	c.828+3A>T	Splice	Het	Mut			Positive

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TABLE 2. Continued

Patient	Gene	Exon	DNA Change	Protein Change	Genotype	Result	PolyPhen Description	PolyPhen Score	Molecular Diagnosis
Patient 14	*PRPH2*	IVS2	c.828+3A>T	Splice	Het	Mut			Positive
Patient 15	*PRPH2*	IVS2	c.828+3A>T	Splice	Het	Mut			Positive
Patient 16	*PRPH2*	IVS2	c.828+3A>T	Splice	Het	Mut			Positive
Patient 17, case 2	*ABCA4*	IVS38	c.5461-10T>C	None	Het	Mut			Unconfirmed
Patient 18	*PRPH2*	2	c.584G>A	p.Arg195Gln	Het	vAR/us	Probably damaging	1	Positive
Cone-rod dystrophy									
Patient 1, dominant	*GUCY2D*	13	c.2512C>T	p.Arg838Cys	Het	Mut			Positive
Patient 2, dominant	*GUCY2D*	13	c.2513G>A	p.Arg838His	Het	Mut			Positive
Patient 3, dominant	*GUCY2D*	13	c.2513G>A	p.Arg838His	Het	Mut			Positive
Patient 4, dominant	*GUCY2D*	13	c.2513G>A	p.Arg838His	Het	Mut			Positive
Patient 5, dominant	*GUCY2D*	13	c.2513G>A	p.Arg838His	Het	Mut			Positive
	CRX	3	c.607T>C	p.Ser213Pro	Het	vAR/us	Probably damaging	0.999	
Patient 6, recessive	*ABCA4*	2	c.156T>G	p.His52Gln	Het	vAR/us	Probably damaging	0.998	Positive
	ABCA4	3	c.161G>A	p.Cys54Tyr	Het	Mut			
	ABCA4	28	c.4169T>C	p.Leu1390Pro	Het	Mut			
Patient 7, recessive	*ABCA4*	16	c.2385C>T	p.Ser795Arg	Het	vAR/us	Probably damaging	0.99	Positive
	ABCA4	IVS40	c.5714+5G>A	Splice	Het	Mut			
Patient 8, recessive	*ABCA4*	42	c.5882G>A	p.Gly1961Glu	Het	Mut			Positive
	ABCA4	45	c.6221G>T	p.Gly2074Val	Het	vAR/us	Probably damaging	1	
Patient 9, recessive	*ABCA4*	IVS42	c.5898+1G<A	Splice	Het	Mut			Positive
	ABCA4	IVS42	c.5899-2delA	Splice	Het	Mut			
Patient 10, recessive	*ABCA4*	5	c.559C>T	p.Arg187Cys	Het	Mut			Positive
	ABCA4	40	c.5645T>C p.Met1882Thr	Het	Mut				
Patient 11, recessive	*ABCA4*	6	c.768G>T	p.Val256Val (abnlspl)	Het	Mut			Positive
	ABCA4	31	c.4577C>T p.Thr1526Met	Het	Mut				
Patient 12, recessive	*ABCA4*	12	c.1622T>C	p.Leu541Pro	Het	Mut			Positive
	ABCA4	21	c.3113C>T	p.Ala1038Val	Het	Mut			
	ABCA4	12	c.1622T>C	p.Leu541Pro	Hom	Mut			
	ABCA4	21	c.3113C>T	p.Ala1038Val	Hom	Mut			
	ABCA4	22	c.3322C>T	p.Arg1108Cys	Het	Mut			
Patient 13, recessive	*ABCA4*	12	c.1622T>C	p.Leu541Pro	Hom	Mut			Positive
	ABCA4	21	c.3113C>T	p.Ala1038Val	Hom	Mut			
Patient 14, recessive	*ABCA4*	13	c.1927G>A	p.Val643Met	Het	Mut			Positive
	ABCA4	24	c.3602T>G	p.Leu1201Arg	Het	Mut			
	ABCA4	36	c.5186T>C	p.Leu1729Pro	Het	Mut			
Patient 15, recessive	*ABCA4*	23	c.3364G>A	p.Glu1122Lys	Het	Mut			Positive
	ABCA4	48	c.6529G>A p.Asp2177Asn	Het	Mut				
Patient 16, recessive	*ABCA4*	35	c.4918C>T	p.Arg1640Trp	Het	Mut			Positive
	ABCA4	28	c.4222T>C	p.Trp1408Arg	Het	Mut			
Patient 17, recessive	*ABCA4*	11	c.1532G>A	p.Arg511His	Het	Mut			Unconfirmed
Patient 18, recessive	*ABCA4*	27	c.3899G>A	p.Arg1300Gln	Het	vAR/us	Benign	0.143	Unconfirmed
Patient 19, recessive	*ABCA4*	13	c.1933G>A	p.Asp645Asn	Het	Mut			Unconfirmed
Patient 20, recessive	*ABCA4*	35	c.4918C>T	p.Arg1640Trp	Het	Mut			Unconfirmed
Patient 21, recessive	*ABCA4*	IVS7	c.859-9T>C	Unknown	Hom	vAR/us	NA	NA	Unconfirmed

TABLE 2. Continued

Patient	Gene	Exon	DNA Change	Protein Change	Genotype	Result	PolyPhen Description	PolyPhen Score	Molecular Diagnosis
Patient 22	ABCA4	42	c.5882G>A	p.Gly1961Glu	Hom	Mut			Positive
Patient 23, recessive	ABCA4	43	c.5917delG	Deletion	Hom	Mut			Positive
Patient 24, recessive	ABCA4	32	c.4661A>G	p.Glu1554Gly	Het	vAR/us	Benign	0.326	Unconfirmed
	ABCA4	30	c.4383G>A						
				p.Trp1461Stop	Het	Mut			
Patient 25, recessive	ABCA4	IVS38	c.5461-10T>C	None	Het	Mut			Positive
	ABCA4	22	c.3259G>A	p.Glu1087Lys	Het	Mut			
Patient 26, recessive	ABCA4	IVS38	c.5461-10T>C	None	Het	Mut			Positive
	ABCA4	42	c.5882G>A	p.Gly1961Glu	Het	Mut			
Patient 27, dominant	GUCY2D	13	c.2513G>A	p.Arg838His	Het	Mut			Positive
Patient 28, recessive, case 5	PRPH2	1	c.514C>T	p.Arg172Trp	Het	Mut			Positive
No specific clinical diagnosis									
Patient 1, case 1	ABCA4	35	c.4919G>A	p.Arg1640Gln	Het	Mut			Positive
	ABCA4	42	c.5882G>A	p.Gly1961Glu	Het	Mut			
	ABCA4	IVS42	c.5898-11G>A	NA	Het	vAR/us	NA	NA	
	ABCA4	IVS48	c.6729+21C>T	NA	Het	vAR/us	NA	NA	

Het, heterozygous; Mut, mutation; vAR, variant; VUS, variant of unknown significance.

were screened for the p.Arg838His mutation in *GUCY2D*, and mutations in the *CRX*, *ELOVL4*, *PRPH2*, and/or *ABCA4* genes. Fifteen CRD families with autosomal recessive inheritance were tested for mutations in the *ABCA4* gene using dideoxy sequencing of the coding region, using the ABCR genotyping microarray (the ABCR400 chip) constructed by APEX technology, or by solid state sequencing.[15,16] Twenty-six patients that were isolated cases and/or lacked a family history for CRD were screened for mutations in *ABCA4*. Ten of the 26 CRD isolated cases also had a differential diagnosis of STGD1 based on early age of onset, presence of central scotoma, and fundus appearance including foveal atrophy, perimacular flecks or a beaten bronze appearance, and increased fundus autoflourescence. These patients underwent additional mutation screening for the *CRX*, *PRPH2*, and *ELOVL4* genes.

When candidate disease-causing variants were identified, several criteria were used to predict their pathogenicity: examination of whether the variant had been previously reported, the bioinformatics score determined by PolyPhen (http://genetics.bwh.harvard.edu/pph2/ [in the public domain]), the association of the genotype with the phenotype, and whether the presence of the variant was consistent with observed pattern of inheritance. When rare (i.e., allele frequency < 0.01) or novel variants of unknown significance (VUS) were present, the variants were analyzed by PolyPhen to predict their potential impact. Novel variants identified as "potentially damaging" support the clinical diagnosis but were validated by segregation analysis to confirm the likelihood that the variants were disease causing. The Berkeley Drosophila Genome Project Splice Site Prediction analysis tool was used to determine whether rare or novel silent variants occurring within the exon were splice altering (http://www.fruitfly.org/seq_tools/splice.html [in the public domain]). Variants identified as "possibly damaging" require additional studies to confirm their involvement in causing pathology. When no mutations were found in the genes associated with the clinical phenotype, then the molecular basis of disease in this patient is unknown, and the molecular diagnosis neither excludes nor supports the clinical diagnosis.

RESULTS

Among the patients registered in the eyeGENE database, 213 unrelated patients were diagnosed with a retinal dystrophy phenotype involving central vision loss. Out of the 213, 6 were diagnosed with LORD, 8 with SFD, 26 with DHRD, 38 with BMD, 74 with PD, 54 with CRD, 6 with both PD and BMD, and 1 with no specific clinical diagnosis.

Genetic Analysis

Late-Onset Retinal Degeneration. No causative mutations were found in the exonic regions of *CTRP5*, demonstrating that coding mutations in *CTRP5* were not the cause of disease in these six patients, and therefore they did not have a positive confirmation of diagnosis (Table 2).

Sorsby Fundus Dystrophy. Of the eight SFD samples, one patient carried a known heterozygous mutation in the *TIMP3* gene and two had the same novel variant (p.Ser38Cys) with the PolyPhen score of 1[1] (Table 2). Thus, 38% of the SFD patients had a positive molecular diagnosis.

Doyne Honeycomb Retinal Dystrophy. Of the 26 patients with DHRD, two patients were heterozygous for known causative mutations in *EFEMP1* leading to a positive molecular diagnosis (Table 2). One of the 26 patients had a novel VUS in *EFEMP1*: a heterozygous variant in the 5′ flanking intronic region of exon 10 (IVS10-14C>T). A disease-causing mutation was identified in only 8% of DHRD patients when testing was limited to only the exonic regions of *EFEMP1*.

Best Macular Dystrophy. Of the 44 patients diagnosed with BMD, causative mutations in *BEST1* were found in 25 patients (Table 1). Twenty patients had known heterozygous mutations; five patients had novel heterozygous variants (p.Leu294Phe, p.Phe84Val, p.Gly83Ala, p.Trp93Arg, p.Trp309Arg) in *BEST1*; each novel variant had a PolyPhen score of 1, predicting that all variants were "probably damaging" mutations. Thus, 57% of the BMD patients had a molecular diagnosis consistent with their clinical diagnosis.

Pattern Dystrophy. Pattern dystrophy is an autosomal dominant inherited disease. Mutations in *PRPH2* (peripherin
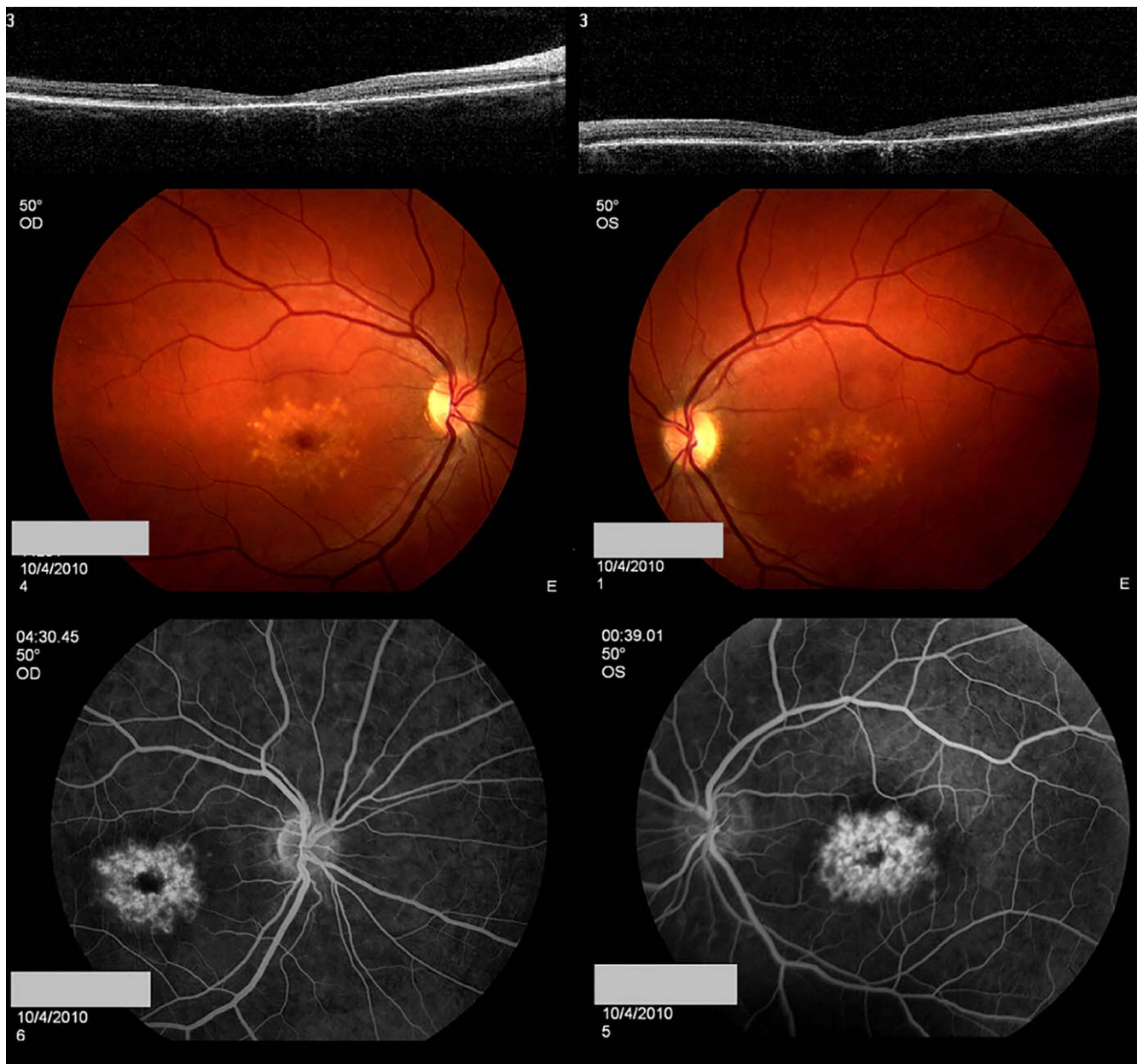
FIGURE 1. Clinical phenotype of case 1. A 44-year-old female with visual acuities of 20/25 OD and 20/60 OS became symptomatic at the age of 35. Submacular yellow deposits are circumscribed and measure approximately 120 μm; some have aggregated and form a pigmented center. Outer retinal atrophy of the foveal region is more prominent in the right eye than the left eye. On fluorescein angiography, these deposits stained with fluorescein, and there was no suggestion of a dark choroid.

2 gene) are known to cause PD. Isolated cases with a PD phenotype were also screened for mutations in *ABCA4* (ATP binding cassette subfamily A, member 4 gene) when no mutations were detected in *PRPH2*. Of the 80 PD patients, 15 showed disease-causing variants in either the *PRPH2* or *ABCA4* gene (Table 2). Thirteen patients were heterozygous for known mutations in *PRPH2*; one additional patient had a novel p.Arg195Gln *PRPH2* variant that was predicted to be "probably damaging," leading to a positive molecular diagnosis. One of the 13 patients was a compound heterozygote for a VUS and a known causative mutation in *PRPH2* (p.Tyr91Asn and p.Ile104Val, respectively) (Table 2). The VUS had a PolyPhen score of 0.909, predicting it to be "probably damaging." Two patients had a single heterozygous mutation in *ABCA4* leading to an unconfirmed

molecular diagnosis, while another patient had two known compound heterozygous *ABCA4* mutations leading to a positive molecular diagnosis (Table 2). One patient had a VUS in *ABCA4* (c.3862+3A>G). This analysis resulted in a positive molecular diagnosis for 19% of the 80 probands with a diagnosis of PD.

Of the 80 patients with PD, six were additionally screened for mutations in *ELOVL4*, one for mutations in *CTRP5*, and six for mutations in *BEST1*. None of the patients with a diagnosis of PD had disease-associated variants in *ELOVL4* or *CTRP5*. One of the patients was found to carry a novel variant in *BEST1*, c.250T>G (p.Phe84Val) (Table 2).

Cone-Rod Dystrophy. Of the 54 CRD patients, 13 showed an autosomal dominant pattern of inheritance. Six patients carried the p.Arg838His or p.Arg838Cys *GUCY2D* mutation
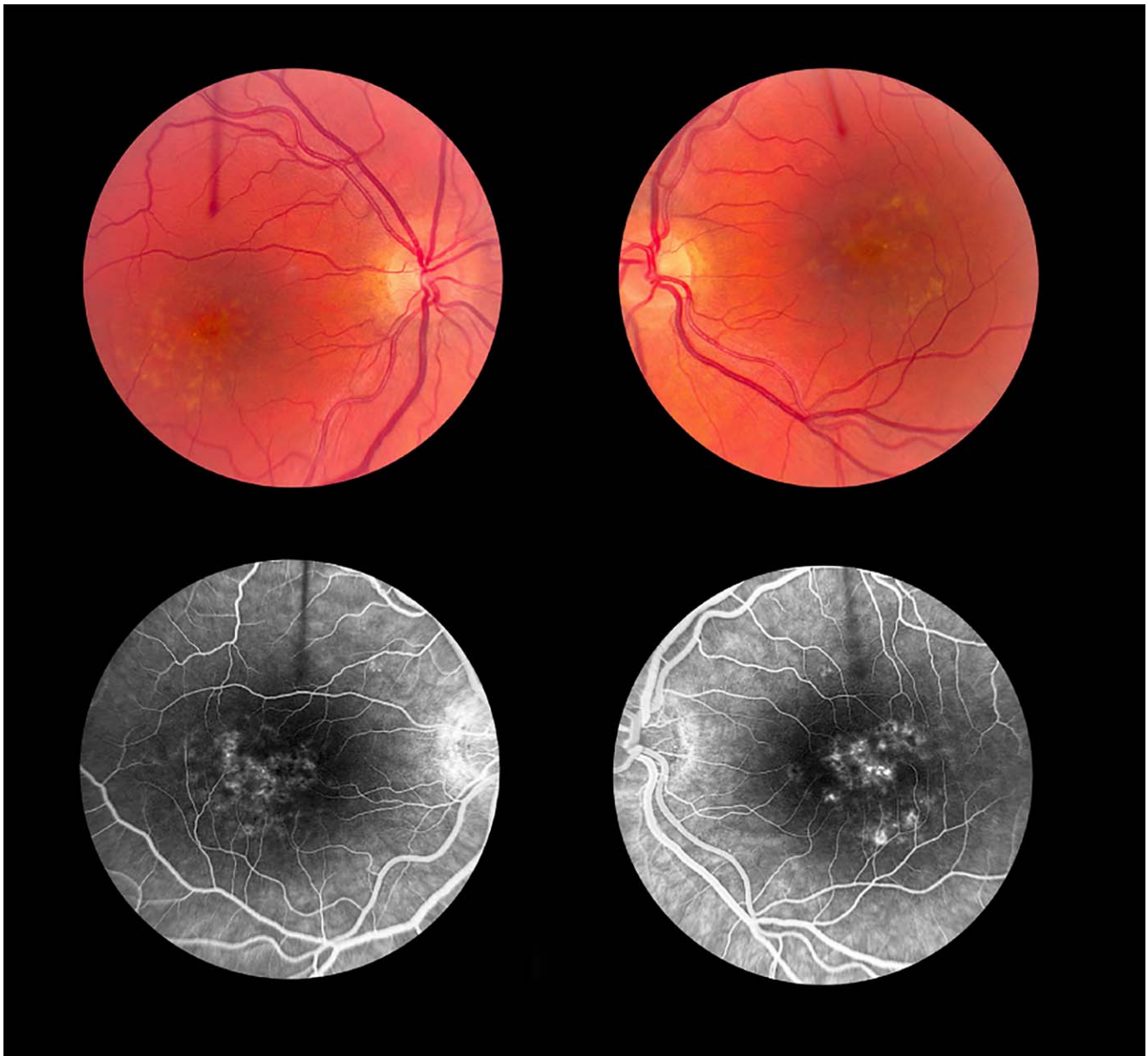
FIGURE 2. Clinical phenotype of case 2. A 39-year-old female with visual acuities of 20/25 OD and 20/200 OS and central scotomas in both eyes became symptomatic at the age of 35. Her OCT showed predominant outer retinal atrophy at the fovea with sub-RPE drusenoid-like deposits. The retinal atrophy is worse in the left eye than the right eye. The yellow circumscribed sub-RPE deposits aggregate as a ring in the parafoveal region. These stained on fluorescein angiography, and there was no suggestion of a dark choroid.

(Table 2). One of the six samples also carried a novel *CRX* variant, p.Ser213Pro (PolyPhen score of 0.998, "probably damaging") (Table 2). These six samples had a positive molecular diagnosis.

Of the 41 CRD patient samples with autosomal recessive inheritance, 21 patient samples carried previously known mutations or novel VUS in *ABCA4*. However, only 15 patients had causative homozygous or compound heterozygous variants in *ABCA4* resulting in a positive molecular diagnosis for recessive CRD. Of these 15, five patients did not have additional family members to test for segregation. The causative mutations included three novel variants: p.His52Gln (PolyPhen score 0.998), p.Ser795Arg (PolyPhen score 0.990), and p.Gly2074Val (PolyPhen score 1). The six cases with unconfirmed molecular diagnoses had variants predicted to be benign, had only a single heterozygous mutation, or had no

mutations. These variants included p.Arg1300Gln and p.Glu1554Gly. The impact of c.859-9T>C detected in one of the patients is unknown, and thus the molecular analysis neither confirms nor excludes the clinical diagnosis in these patients (Table 2). In summary, 41% of the combined dominant and recessive CRD patient samples had a positive molecular diagnosis that confirmed their clinical diagnosis.

Phenotype and Genotype of Six Selected Patients With Late-Onset Retinal Pathology and Drusen

Case 1 (Multiple Diagnoses, No. 1). A 44-year-old female with a visual acuity of 20/25 OD and 20/60 OS became symptomatic at the age of 35. Submacular yellow deposits of approximately 120 μm, some aggregated with a pigmented
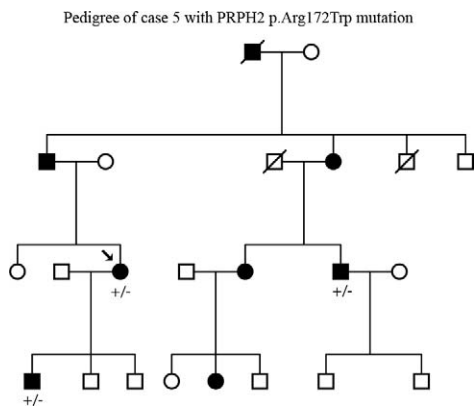
Pedigree of case 5 with PRPH2 p.Arg172Trp mutation

FIGURE 3. Pedigree of case 5. Segregation of the *PRPH2* mutation at c.514C>T (p.Arg172Trp) with disease.

Pedigree of case 6 with PRPH2 p.Tyr141Cys mutation

FIGURE 4. Pedigree of case 6. Segregation of the *PRPH2* mutation at c.422A>G (p.Tyr141Cys) with disease. *Striped background* designates family members afflicted with hearing loss, and *solid black* designates family members affected by retinal degeneration. *Question mark* designates status unknown.

center, were observed. Retinal atrophy of the foveal region was more prominent in the right eye than the left eye (Figs. 1A, 1B). On fluorescein angiography, the deposits stained with fluorescein, and there was no suggestion of a dark choroid (Figs. 1C, 1D). The patient was enrolled with the potential diagnoses of DHCD, STGD1, or SFD.

Analysis of *EFEMP1* did not identify causative mutations. Subsequent analysis of *ABCA4* revealed two previously reported heterozygous mutations, c.4919G>A (p.Arg1640Gln) and c.5882G>A (p.Gly1961Gln), and six additional heterozygous VUS. Four of these novel changes were synonymous variants and were predicted to not cause splice alterations. Two additional novel variants were located in the intronic region (c.5898-11G>A and c.6729+21C>T) (Table 2).

Case 2 (PD, No. 17). A 39-year-old female with a visual acuity of 20/25 OD and 20/200 OS, with central scotomas in both eyes, became symptomatic at age 35. Her optical coherence tomography (OCT) showed predominant outer retinal atrophy at the fovea with sub-RPE drusenoid deposits (Figs. 2A, 2B). The retinal atrophy was worse in the left eye than the right eye (Figs. 2C, 2D). The yellow circumscribed sub-RPE deposits aggregated as a ring in the parafoveal region and stained on fluorescein angiography, and there was no suggestion of a dark choroid (Figs. 2E, 2F). The 24/2 Humphrey visual field test showed central visual field deficits in both eyes.

This patient was enrolled as having STGD1, PD, SFD or DHRD. Analysis of *ABCA4*, *ELOVL4*, *PRPH2*, and *TIMP3* did not reveal the presence of known mutations. A single heterozygous known mutation, c.5461-10T>C, was detected in the *ABCA4* gene (Table 2).

Case 3 (BMD, No. 25). An 81-year-old male with a visual acuity of 20/200 OD, counting finger OS, and central scotoma in both eyes became symptomatic at age 47. He had normal full-field electroretinography (ERG) findings with retinal atrophy in the fovea of both eyes. This patient was enrolled as having BMD.

This patient was screened for mutations in *PRPH2* and *BEST1*. Causative changes were not detected in *PRPH2*. Analysis of the *BEST1* revealed two homozygous novel silent variants, c.1557C>T (p.Ser519Ser) and c.1608T>C (p.Thr536Thr) in exon 10, and one novel heterozygous missense variant, c.250T>G (p.Phe84Val) in exon 4, with the PolyPhen score of 1. Neither of the silent variants was predicted to alter splicing (Table 2).

Case 4 (PD, No. 13). A female with a visual acuity of 20/20 OD, 20/30 OS presented with visual symptoms at age 39. She had yellow sub-RPE deposits in both maculas. She was enrolled with a diagnosis of BMD or PD. Mutation analysis did not detect
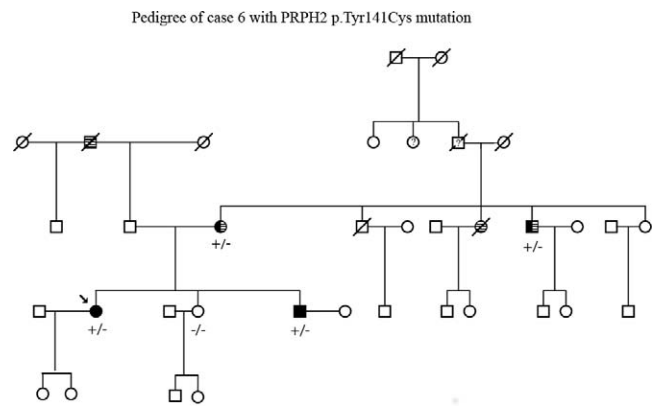
causative mutations in *BEST1*, while a previously reported splice site mutation c.828+3A>T was detected in *PRPH2* in the heterozygous state (Table 2).

Case 5 (CRD, No. 28). A 63-year-old woman with a visual acuity of 20/40 OU and central scotoma in both eyes became symptomatic at age 46. Her full-field ERG showed reduced photopic and scotopic responses, and she exhibited signs of macular degeneration on examination.

This patient was enrolled with a diagnosis of dominant CRD. Initially the coding sequence of the *CRX* gene and targeted testing of codon Arg838 of *GUCY2D* were performed. When both these tests came up negative, *PRPH2* was screened, and a heterozygous mutation, c.514C>T (p.Arg172Trp) that segregated with disease in the family was found (Table 2; Fig. 3).

Case 6 (PD, No. 5). An 82-year-old man with visual acuities of 20/20 OD and 20/25 OS became symptomatic at age 40. He had signs of macular degeneration on examination. The patient and two additional affected family members (a sister and a niece) were enrolled in eyeGENE with a diagnosis of PD. Analysis of *CRX* and *ELOVL4* did not detect causative mutations, but *PRPH2* testing revealed a heterozygous mutation, c.422A>G (p.Tyr141Cys), in the *PRPH2* gene. The *PRPH2* mutation was also observed in both affected relatives (Table 2; Fig. 4).

DISCUSSION

Of the 213 samples, 55 patients (26%) had known causative mutations and 13 (6%) patients had VUS that were possibly pathogenic (Table 3). Best macular dystrophy had the highest success rate (57% of patients) for molecular diagnosis, likely contributed to by the relatively limited variation in phenotype: abnormal electro-oculography (EOG) and vitelliform lesions. The lowest rate of success was found in LORD patients, with none of the six patients having a positive molecular diagnosis. Late-onset retinal degeneration, an extremely rare disease with a phenotype that overlaps with many other retinal dystrophies including the common AMD, is often misdiagnosed.[17] So far, only one mutation in *CTRP5/C1QTNF5* has been reported in families of European origin.[9] Lack of *CTRP5* gene mutations in patients diagnosed with LORD may indicate involvement of other genes or the presence of mutations in the unscreened regions of the genes (introns or the promoter region).

TABLE 3. Mutations or Unknown Variants Detected in Patients With Central Vision Loss

Gene	Exon	DNA Change	Protein Change	Genotype	Result	PolyPhen Description	PolyPhen Score	Frequency*	Variant ID
Late-onset retinal degeneration									
CTRP5	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sorsby fundus dystrophy									
TIMP3	1	c.113C>G	p.Ser38Cys	Het	vAR/us	Probably damaging	1	2	
TIMP3	5	c.610A>T	p.Ser204Cys	Het	Mut			1	CM941325/ rs137853298
Doyne honeycomb dystrophy									
EFEMP1	9	c.1033C>T	p.Arg345Trp	Het	Mut			2	CM990504
EFEMP1	IVS10	c.IVS10-14C>T	None	Het	vAR/us	NA	NA	1	
Best macular dystrophy									
BEST1	2	c.28G>A	p.Ala10Thr	Het	Mut			1	CM982017
BEST1	2	c.47C>T	p.Ser16Phe	Het	Mut			1	CM010520
BEST1	2	c.72G>T	p.Trp24Cys	Het	Mut			1	CM982018
BEST1	3	c.240C>A	p.Phe80Leu	Het	Mut			2	CM004423
BEST1	4	c.248G>C	p.Gly83Ala	Het	vAR/us	Probably damaging	1	1	
BEST1	4	c.277T>C	p.Trp93Arg	Het	vAR/us	Probably damaging	1	1	
BEST1	4	c.279G>C	p.Trp93Cys	Het	Mut			1	rs28940273/ CM982021
BEST1	6	c.652C>T	p.Arg218Cys	Het	Mut			2	CM982023
BEST1	6	c.680A>G	p.Tyr227Cys	Het	Mut			1	CM982024
BEST1	6	c.741G>A	p.Arg218His	Het	Mut			2	CM003486
BEST1	7	c.727G>A	p.Ala243Thr	Het	Mut			2	CM004434
BEST1	7	c.728C>T	p.Ala243Val	Het	Mut			2	rs28940570/ CM00841
BEST1	8	c.880C>T	p.Leu294Phe	Het	vAR/us	Probably damaging	1	1	
BEST1	8	c.887A>G	p.Asn296Ser	Het	Mut			1	CM010524
BEST1	8	c.903T>G	p.Asp301Glu	Het	Mut			2	CM991243
BEST1	8	c.910G>A	p.Asp304Asn	Het	Mut			1	CM024219
BEST1	8	c.925T>C	p.Trp309Arg	Het	vAR/us	Probably damaging	1	1	
BEST1	8	c.929T>C	p.Ile310Thr	Het	Mut			1	CM000843
BEST1	4	c.250T>G	p.Phe84Val	Het	vAR/us	Probably damaging	1	1	
Pattern dystrophy									
ABCA4	6	c.634C>T	p.Arg212Cys	Het	Mut			1	rs61750200
ABCA4	17	c.2588G>C	p.Gly863Ala	Het	Mut			1	CM970003/ rs76157638
ABCA4	IVS26	c.3862+3A>G	Abnormal splicing	Het	vAR/us			1	NA
ABCA4	30	c.4469G>A	p.Cys1490Tyr	Het	Mut			1	CM990056/ rs61751402
ABCA4	IVS38	c.5461-10T>C	None	Het	Mut			1	CS057513
PRPH2	1	c.271T>A	p.Tyr91Asn	Het	vAR/us	Probably damaging	.909	1	
PRPH2	1	c.310-313del(AT)	p.Ile104Val	Het	Mut			1	NA/Deletion
PRPH2	1	c.422A>G	p.Tyr141Cys	Het	Mut			2	CM010125/ rs61755781
PRPH2	1	c.515G>A	p.Arg172Gln	Het	Mut			1	CM930637/ rs61755792
PRPH2	2	c.583C>T	p.Arg195Stop	Het	Mut			1	CM032999
PRPH2	2	c.629C>G	p.Pro210Arg	Het	Mut			1	CM941210
PRPH2	2	c.635G>C	p.Ser212Thr	Het	Mut			1	CM971289/ rs61755801
PRPH2	2	c.683C>T	p.Thr228Ile	Het	Mut			1	TMP_ESP_6_ 42672248
PRPH2	2	c.708C>G	p.Tyr236Stop	Het	Mut			1	rs61755813
PRPH2	IVS2	c.828+3A>T	Splice	Het	Mut			4	CS010139
PRPH2	2	c.584G>A	p.Arg195Gln	Het	vAR/us	Probably damaging	1	1	

TABLE 3. Continued

Gene	Exon	DNA Change	Protein Change	Genotype	Result	PolyPhen Description	PolyPhen Score	Frequency*	Variant ID
Cone-rod dystrophy									
ABCA4	2	c.156T>G	p.His52Gln	Het	vAR/us	Probably damaging	0.998	1	
ABCA4	3	c.161G>A	p.Cys54Tyr	Het	Mut			1	CM990012/ rs150774447
ABCA4	28	c.4169T>C	p.Leu1390Pro	Het	Mut			1	CM014810/ rs61752430
ABCA4	16	c.2385C>T	p.Ser795Arg	Het	vAR/us	Probably damaging	0.99	1	
ABCA4	IVS40	c.5714+5G>A	Splice	Het	Mut			1	CS982057
ABCA4	27	c.3899G>A	p.Arg1300Gln	Het	vAR/us	Benign	0.143	1	
ABCA4	32	c.4661A>G	p.Glu1554Gly	Het	vAR/us	Benign	0.326	1	
ABCA4	30	c.4383G>A	p.Trp1461Stop	Het	Mut			1	Stop/NA
ABCA4	IVS38	c.5461-10T>C	None	Het	Mut	NA	NA	2	CS057513
ABCA4	22	c.3259G>A	p.Glu1087Lys	Het	Mut			1	CM970008/ rs61751398
ABCA4	42	c.5882G>A	p.Gly1961Glu	Het	Mut			2	CM970016/ rs1800553
ABCA4	45	c.6221G>T	p.Gly2074Val	Het	vAR/us	Probably damaging	1	1	
ABCA4	IVS42	c.5898+1G<A	Splice	Het	Mut			1	CS011524
ABCA4	IVS42	c.5899-2delA	Splice	Het	Mut			1	rs3112831
CRX	3	c.607T>C	p.Ser213Pro	Het	vAR/us	Probably damaging	0.999	1	
ABCA4	5	c.559C>T	p.Arg187Cys	Het	Mut			1	COSM913472
ABCA4	40	c.5645T>C	p.Met1882Thr	Het	Mut			1	rs4147830
ABCA4	6	c.768G>T	p.Val256Val (abnlspl)	Het	Mut			1	CM990057/ rs61750152
ABCA4	31	c.4577C>T	p.Thr1526Met	Het	Mut			1	rs62645944
ABCA4	11	c.1532G>A	p.Arg511His	Het	Mut			1	rs140482171
ABCA4	12	c.1622T>C	p.Leu541Pro	Het	Mut			1	CM990022/ rs61751392
ABCA4	21	c.3113C>T	p.Ala1038Val	Het	Mut			1	CM970006/ rs61751374
ABCA4	12	c.1622T>C	p.Leu541Pro	Hom	Mut			2	CM990022/ rs61751392
ABCA4	21	c.3113C>T	p.Ala1038Val	Hom	Mut			2	CM970006/ rs61751374
ABCA4	22	c.3322C>T	p.Arg1108Cys	Het	Mut			1	CM990039/ rs61750120
ABCA4	13	c.1927G>A	p.Val643Met	Het	Mut			1	CM014293/ rs61749417/ rs143548435
ABCA4	24	c.3602T>G	p.Leu1201Arg	Het	Mut			1	CM990042/ rs61750126
ABCA4	36	c.5186T>C	p.Leu1729Pro	Het	Mut			1	CM990062/ rs61750567
ABCA4	13	c.1933G>A	p.Asp645Asn	Het	Mut			1	rs617494181933
ABCA4	23	c.3364G>A	p.Glu1122Lys	Het	Mut			1	CM990041
ABCA4	48	c.6529G>A	p.Asp2177Asn	Het	Mut			1	CM970023/ rs1800555
ABCA4	35	c.4918C>T	p.Arg1640Trp	Het	Mut			2	CM983728/ rs61751404
ABCA4	28	c.4222T>C	p.Trp1408Arg	Het	Mut			1	CM990048/ rs61750135
GUCY2D	13	c.2512C>T	p.Arg838Cys	Het	Mut			1	rs61750172
GUCY2D	13	c.2513G>A	p.Arg838His	Het	Mut			5	CM012606/ rs61750173
ABCA4	IVS7	c.859-9T>C	Unknown	Hom	vAR/us	NA	NA	1	
ABCA4	42	c.5882G>A	p.Gly1961Glu	Hom	Mut			1	CM970016/ rs1800553
ABCA4	43	c.5917delG	Deletion	Hom	Mut			1	RISN_ABCR: c.5917delG

TABLE 3. Continued

Gene	Exon	DNA Change	Protein Change	Genotype	Result	PolyPhen Description	PolyPhen Score	Frequency*	Variant ID
PRPH2	1	c.514C>T	p.Arg172Trp	Het	Mut			1	CM930639
No specific clinical diagnosis									
ABCA4	35	c.4919G>A	p.Arg1640Gln	Het	Mut			1	CM003577
ABCA4	42	c.5882G>A	p.Gly1961Glu	Het	Mut			1	CM970016/ rs1800553
ABCA4	IVS42	c.5898-11G>A	NA	Het	vAR/us	NA	NA	1	
ABCA4	IVS48	c.6729+21C>T	NA	Het	vAR/us	NA	NA	1	

* Frequency signifies number of times a mutation is observed within the data set.

Six patients with late-onset retinal pathology and drusen had well-characterized clinical data. Case 1 had two known mutations, c.4919 G>A (p.Arg1640Gln) and c.5882G>A (p.Gly1961Glu), in exons 35 and 42 of *ABCA4*. The presence of these two mutations in the compound heterozygous state in patients with a diagnosis of SD and CRD has been reported.[18,19] Involvement of *ABCA4* in causing pathology in this patient could not be confirmed, since additional family members were unavailable to evaluate if the two mutations occurred in the cis or trans configuration. Case 2 also had LORD with drusenoid deposits and carried a single *ABCA4* mutation in the heterozygous state. Although mutations in *ABCA4* have been reported to be associated with LORD, the lack of data on additional family members and the absence of the second mutation in case 2 limited the ability to evaluate the association between genotype and phenotype. Three additional patients (cases 4, 5, and 6) had heterozygous mutations in *PRPH2* (Table 2). Two of these patients (cases 5 and 6) had additional affected family members, and the mutations segregated with disease (Figs. 3, 4). These observations are consistent with earlier reports on association of *PRPH2* mutations with a wide range of retinal dystrophy phenotypes including PD, late-onset drusen, and macular dystrophy.[20] Overall, mutations in the *ABCA4*, *PRPH2*, and *BEST1* genes were found in the six patients with a LORD phenotype. Selection of *PRPH2* and *BEST1* genes for testing may result in a higher success rate in providing a positive molecular diagnosis for patients with late-onset retinal pathology and positive family history of RD, whereas sporadic cases or patients with no family history are more likely to carry mutations in *ABCA4*.

The RD phenotype involving central vision loss is associated with a group of genes implicated in a broad range of overlapping clinical symptoms. In the current study, six patients were diagnosed with both BMD and PD; one patient (case 4) was found to carry a *PRPH2* mutation, confirming the PD diagnosis, and a second patient (case 3) was found carrying a VUS in the *BEST1* gene, supporting the BMD diagnosis. One patient (case 5) diagnosed with autosomal dominant CRD carried a *PRPH2* mutation. Another patient (case 6) with late-onset PD also carried a *PRPH2* mutation. One patient (case 1) with a primary diagnosis of DHRD and secondary diagnoses of STGD1 and SFD carried two heterozygous mutations in the *ABCA4* gene. These cases demonstrate the heterogeneity in clinical phenotype of LORD and the challenge in establishing genotype–phenotype associations in retinal dystrophies. Analysis of a larger sample set with well-characterized phenotype data will assist in understanding the association between phenotypes and specific genotypes in known and novel genes. Inconsistencies in patient diagnosis from referring clinicians may have contributed to the discrepancies in findings since the genetic screening strategy first targeted genes that were associated with specific phenotypes. The small cohort size of patients with diseases such as SFD and LORD, eight and six, respectively, limited the ability to draw any significant conclusions on the outcome of genetic analysis. Furthermore, the lack of information on VUS also affected the ability to establish definitive molecular diagnosis. Although PolyPhen analysis was performed on each novel mutation, the results are computational predictions that require biological or experimental confirmation. Differing methodologies used for diagnostic genetic screening were also a limitation in this study. Some samples were screened for mutations in all the exons of the genes of interest, while others were screened for mutations in only a subset of genetic regions. Sequential genetic screening that examines the most common mutations first, followed by examination of the most common disease-associated genes and finally the less common disease-associated genes or all genes, is cost-effective and efficient if a causal mutation is identified. However, this strategy does not provide uniform genetic information on all samples. With the rapid decrease in sequencing costs, sequencing of whole genomes, exomes, or custom capture of all known retinal disease genes is currently the best approach to identifying the genetic basis for retinal diseases.

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