Whole Exome Sequencing Reveals *GUCY2D* as a Major Gene Associated With Cone and Cone–Rod Dystrophy in Israel

Csilla H. Lazar,^{1,2} Mousumi Mutsuddi,^{1,3} Adva Kimchi,⁴ Lina Zelinger,^{1,4} Liliana Mizrahi-Meissonnier,⁴ Devorah Marks-Ohana,⁴ Alexis Boleda,¹ Rinki Ratnapriya,¹ Dror Sharon,⁴ Anand Swaroop,¹ and Eyal Banin^{1,4}

¹Neurobiology-Neurodegeneration & Repair Laboratory, National Eye Institute, National Institutes of Health, Bethesda, Maryland, United States

²Molecular Biology Center, Interdisciplinary Research Institute on Bio-Nano Sciences, Babes-Bolyai-University, Cluj-Napoca, Romania

³Department of Molecular and Human Genetics, Banaras Hindu University, Varanasi, India

⁴Department of Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Correspondence: Anand Swaroop, National Eye Institute, NIH Building 6/338, 6 Center Drive, Bethesda, MD 20892-0610, USA;

swaroopa@nei.nih.gov.

Eyal Banin, Department of Ophthalmology, Hadassah-Hebrew University Medical Center, POB 12000 Jerusalem 91120, Israel;

banine@mail.huji.ac.il.

Dror Sharon, Department of Ophthalmology, Hadassah-Hebrew University Medical Center, POB 12000 Jerusalem 91120, Israel; dror.sharon1@gmail.com.

Submitted: September 8, 2014

Accepted: November 30, 2014

Citation: Lazar CH, Mutsuddi M, Kimchi A, et al. Whole exome sequencing reveals *GUCY2D* as a major gene associated with cone and cone-rod dystrophy in Israel. *Invest Ophthalmol Vis Sci*. 2015;56:420–430. DOI:10.1167/iovs.14-15647

PURPOSE. The Israeli population has a unique genetic make-up, with a high prevalence of consanguineous marriages and autosomal recessive diseases. In rod-dominated phenotypes, disease-causing genes and mutations that differ from those identified in other populations often are incurred. We used whole exome sequencing (WES) to identify genetic defects in Israeli families with cone-dominated retinal phenotypes.

METHODS. Clinical analysis included family history, detailed ocular examination, visual function testing, and retinal imaging. Whole exome sequencing, followed by segregation analysis, was performed in 6 cone-dominated retinopathy families in which prior mutation analysis did not reveal the causative gene. Based on the WES findings, we screened 106 additional families with cone-dominated phenotypes.

RESULTS. The WES analysis revealed mutations in known retinopathy genes in five of the six families: two pathogenic mutations in the *GUCY2D* gene in three families, and one each in *CDHR1* and *C8orf37*. Targeted screening of additional cone-dominated families led to identification of *GUCY2D* mutations in four other families, which included two highly probable novel disease-causing variants.

CONCLUSIONS. Our study suggested that *GUCY2D* is a major cause of autosomal dominant cone and cone-rod dystrophies in Israel; this is similar to other Caucasian populations and is in contrast with retinitis pigmentosa (primary rod disease), where the genetic make-up of the Israeli population is distinct from other ethnic groups. We also conclude that WES permits more comprehensive and rapid analyses that can be followed by targeted screens of larger samples to delineate the genetic structure of retinal disease in unique population cohorts.

Keywords: next generation sequencing, photoreceptor degeneration, inherited blindness

The Israeli population is rather unique in that it includes a number of ethnic groups with high prevalence of consanguineous marriages. These groups, including Jews of different origins, Arab Muslim, Arab Christian, Bedouin, and Druze subpopulations, differ in their religion and geographic/cultural origin. Therefore, the prevalence of hereditary diseases, particularly with an autosomal recessive (AR) pattern of transmission, is high and novel genes as well as novel mutations in known genes often are identified (Israeli national genetic database, available in the public domain at <http://server.goldenhelix.org/israeli/>).¹ The same holds true for inherited retinal degenerations, with founder mutations in specific subpopulations often being the cause of disease.^{2–6} In rod-dominated degenerations, the most prevalent is the retinitis pigmentosa (RP) phenotype. Autosomal dominant (AD) inheritance of RP in the Israeli and Palestinian populations appears to be infrequent and currently is estimated at 7.3% in our

cohort of 547 families (Sharon and Banin, unpublished data) versus 30% to 40% in other populations.⁷ In contrast, AR-RP often is detected because of consanguinity and limited mixing among different subpopulations, with the most frequent cause among Jews being *FAM161A* gene mutations, which are rare elsewhere.^{6,8} Thus, modes of inheritance and genetic causes of disease in Israel differ from those described in the European and North American populations.

To evaluate the hypothesis that cone-dominated retinal disease in Israel has a distinct genetic architecture compared to the Caucasian population, we investigated the cause of cone dystrophies (CD) and cone-rod dystrophies (CRD), phenotypes minimally studied in Israeli cohorts. Cone dystrophy is characterized by early loss of cone photoreceptors that manifests in deterioration of visual acuity, color vision abnormalities, photo aversion, and appearance of central scotomas. Cone-rod dystrophy presents additional rod photo-

receptor dysfunction that leads to night-blindness and loss of peripheral vision later in the disease process.⁹ The severity and age of onset of both diseases often vary among patients, but they usually are progressive in nature.¹⁰ Also, CD and CRD can be distinguished by the analysis of electroretinograms (ERG), which reveal substantially abnormal cone response in both disorders and an additional aberrant rod response in CRD.¹¹

The disorders of CD and CRD are clinically and genetically heterogeneous conditions with an estimated prevalence of 1 in 30,000 to 40,000 individuals.¹² Autosomal dominant, AR, and X-linked recessive (XL) modes of inheritance have been reported. To date, mutations in at least 30 genes have been implicated in cone-dominated retinal disease and a number of additional loci have been mapped (RetNet; available in the public domain at <https://sph.uth.edu/retnet/>). However, reported genes/mutations explain only a fraction of the cases observed. It should be noted that mutations in five genes can explain more than 90% of cases in achromatopsia, a cone disorder that is less progressive than CD and CRD. In contrast, the currently known genes account for less than 25% of AR and AD CD/CRD cases.⁹ Among X-linked CD/CRD patients, more than 50% are due to mutations in the *RPGR* gene. As part of the genetic heterogeneity observed among retinal disorders, mutations in many genes associated with cone and cone-rod dystrophies also are involved in other retinal disease phenotypes, such as RP, Leber congenital amaurosis (LCA), and congenital stationary night blindness (CSNB).^{11,12}

In the present study, we focused on the identification of genetic defects in Israeli families with primary cone disease and took advantage of recently developed next generation sequencing-based genome wide analysis. Whole exome sequencing (WES) of 6 families of Israeli origin resulted in the detection of mutations in the retinal guanylate cyclase 2D (*GUCY2D*) gene in three families, prompting us to perform targeted genetic screening in 106 additional families. Our studies demonstrated *GUCY2D* as an important cause of CD/CRD in the Israeli population, in concordance with its prevalence in other ethnic groups.

METHODS

Patient Ascertainment and Clinical Assessment

An informed consent that adhered to the tenets of the Declaration of Helsinki was signed by all participants of the present study, which was approved by the Hadassah Medical Center institutional review board. For the current study, we selected families with nonsyndromic, cone-dominated autosomal disease from our cohort of Israeli and Palestinian patients manifesting hereditary retinopathies. DNA was extracted from the index patients as well as from other affected and unaffected family members using the FlexiGene DNA kit (Qiagen, Venlo, The Netherlands). A detailed clinical evaluation was performed that included family history, a full ophthalmologic exam, visual function testing, and retinal imaging, as described previously.⁵ Briefly, International Society for Clinical Electrophysiology of Vision (ISCEV) standard full-field electroretinography (ffERG) was performed using monopolar corneal electrodes (Henkes type; Medical Workshop B.V., Groningen, The Netherlands) and a computerized system (UTAS 3000; LKC, Gaithersburg, MD, USA). Cone responses to 30-Hz flashes of white light were acquired under a background light of 21 cd/m². Scotopic responses, including a rod response to a dim blue flash and a mixed cone-rod response to a standard white flash, were acquired after 30 to 45 minutes of dark adaptation. Between 2 and 4 sets of responses were recorded in each condition to verify repeatability. All ERG responses were filtered at 0.3 to

500 Hz, and signal averaging was applied. Color vision was evaluated using the Farnsworth D-15 panel. Visual field testing was performed using a Goldmann kinetic perimeter or a Humphrey field analyzer, and microperimetry (MP) under direct visualization of the fundus was done on a Macular Integrity Assessment (MAIA) system (CenterVue SpA, Padova, Italy). Color fundus (CF) photos were obtained using a Zeiss fundus camera (model FF450PLUS; Carl Zeiss Meditec, Dublin, CA, USA) or an OPTOS widefield imaging system (model 200Tx; OPTOS Plc, Scotland, UK). Optical coherence tomography (OCT) and infrared imaging were acquired using the Spectralis system (Heidelberg Engineering, Heidelberg, Germany). Fundus autofluorescence (FAF) imaging was performed on the Spectralis and/or OPTOS systems described above.

Genetic Analyses

Whole genome single nucleotide polymorphism (SNP) analysis was performed on the index case (MOL0858 II:1) and the affected sister (MOL0858 II:2) of family MOL0858 using Affymetrix (Santa Clara, CA, USA) SNP microarray platform 6.0. The array data were analyzed using HomozygosityMapper (available in the public domain at <http://www.homozygositymapper.org/>), and homozygous regions were defined as those harboring at least 3900 consecutive homozygous SNPs. Specific founder mutations were screened by Sanger sequencing and were found to be negative. Sanger sequencing was used for targeted mutation screening of exons 13-14 in the *GUCY2D* gene with a reported primer set.¹³ The primer sequences were: forward, GTAGATGA ATGGTGGCAGCG; reverse, GATTGGGCAGGTAGGCTAGG.

Exome Library Construction

Whole exome capture was performed with 3 µg of genomic DNA using Agilent SureSelect Human All Exon V5 Kit (Agilent Technologies, Santa Clara, CA, USA), according to manufacturer's instructions. Captured libraries were converted into clonal clusters using Cluster Station 408 (Illumina, San Diego, CA, USA). Single-end, 110 bp sequence reads were generated by Genome Analyzer IIX (Illumina).

Data Analysis and Validation

Quality score assessment at each nucleotide position was performed using FastQC (available in the public domain at <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>) quality control tool. Reads were mapped against the human reference genome NCBI build 37 (hg19) using Genomatix Mining Station. Genomatix software incorporates SAMtools¹⁴ for removal of duplicates and nonunique alignment, as well as for identification of single nucleotide variants and small insertion-deletions. Variants were annotated using ANNOVAR.¹⁵ Prioritization was performed based on the functional consequence of the observed change (stop gain/loss > missense > splice site), and frequency information was checked in the dbSNP137 (available in the public domain at <http://www.ncbi.nlm.nih.gov/SNP/>), 1000 Genome Project (available in the public domain at <http://www.1000genomes.org/>), and Exome Sequencing Project (ESP) database (available in the public domain at <http://evs.gs.washington.edu/EVS/>). The pathogenicity of candidate variants was evaluated using bioinformatic tools - PolyPhen2 (available in the public domain at <http://genetics.bwh.harvard.edu/pph2/>), MutationTaster (available in the public domain at <http://www.mutationtaster.org/>), SIFT (available in the public domain at <http://sift.jcvi.org/>), Provean (available in the public domain at <http://provean.jcvi.org/index.php>), and VarioWatch (available in the

public domain at <http://genepipe.ncgm.sinica.edu.tw/variowatch/main.do>). The sequence position and coverage of variants were visualized with Integrative Genomics Viewer.¹⁶ Extraction of variants that segregate according to the presumed mode of inheritance was performed using Partek Genomics Suite, version 6.11 (Partek, Inc., St. Louis, MO, USA) and JMP 10 (SAS Institute, Inc., Cary, NC, USA) statistical analysis software. All variants of interest were validated using conventional Sanger sequencing and were found to be negative in a set of 250 Israeli exome samples. Where available, segregation analysis was performed on DNA samples from additional family members. Interspecies conservation of amino acid sequence positions affected by the identified novel genetic variants was generated with the ClustalW aligner of MacVector software, version 12.7.5 (MacVector, Inc., Cary, NC, USA).

RESULTS

To determine the genetic cause of retinal degenerations with cone-dominated phenotypes, whole exome sequencing was performed on a total of 20 individuals from 6 unrelated Israeli families (Fig. 1). In three of the families (MOL0490, MOL0859, MOL1103), disease segregation followed an apparent AD inheritance pattern. Two families (MOL0056, MOL0858) were consanguineous with presumably AR inheritance, and the sixth (MOL0048) was suggestive of either AR or XL inheritance. Exome sequencing was performed because our initial molecular genetic analysis, which included screening of the AR families for founder mutations prevalent among other families of the same ethnic origin, did not reveal any causal mutations. Among the AD families, no disease-causing mutations have yet been reported in the Israeli population. For WES, we selected 3 to 4 individuals from each family, including one unaffected control. Our analysis of variants led to the identification of four previously reported mutations in three retinal disease genes: *GUCY2D* mutations in all three AD families, and *CDHR1* and *C8orf37* mutations in the presumed AR families (Table 1). All variants were validated by Sanger sequencing and segregated with the disease phenotype within the respective family. Extensive analysis of the variants identified from the exome data did not reveal a potential causative mutation that showed segregation with disease in family MOL0048 (Fig. 1C).

Dominant Families

The three dominant families harbored heterozygous *GUCY2D* missense mutations that have been reported previously (Table 1): c.2512C>T (p.R838C)¹⁷ in families MOL0490 and MOL1103, and c.2513G>A (p.R838H)¹⁸ in family MOL0859. This result suggested that *GUCY2D* mutations, although not previously described in Israeli patients, might be a significant cause of AD cone-dominated disease. Therefore, we performed targeted sequencing of *GUCY2D* exons 13 and 14 in 106 additional Israeli index cases (including 23 with AD cone-dominated disease, 50 isolate cases with cone-dominated disease and 33 with ADRP) selected from our cohort of over 1300 families with hereditary retinal disease. We identified rare heterozygous sequence changes in exon 13 in two AD cone-dominated families (MOL0083, MOL0508) and two of the isolate cases (MOL0248, MOL0430; Fig. 1A, Table 1): c.2512C>T (p.R838H) in MOL0083 and MOL0248, c.2521G>A (p.E841K) in family MOL0508, and c.2538G>C (p.K846N) in family MOL0430. In five of the seven families with AD inheritance, the mutations altered the amino acid residue 838. Two other variants (p.E841K and p.K846N) were not detected in public databases (1000 Genome Project,

dbSNP, ESP, and a local database of 250 Israeli exomes) and are predicted to be pathogenic by online mutation prediction software programs (PolyPhen2, SIFT, Proven, MutationTaster, VarioWatch, see Supplementary Table S1). These two affected residues are highly conserved across species and are part of the dimerization domain of the protein (Fig. 2B).

Clinically, affected patients from the seven families presented phenotypes that are largely within the spectrum reported for heterozygous (AD) *GUCY2D*-associated disease (Table 2, Fig. 3).^{13,19} Briefly, the onset of disease occurred between childhood and early adulthood, with a progressive nature. Visual acuity was reduced in all patients. In two children (4–5 years old), acuities of 0.5 and 0.7 were measured, but above age 20 acuities ranged from 0.4 to finger counting at 1 m. High myopia was evident in almost all patients; interestingly, the only emmetropic patient (MOL0859 II:1) maintained the best visual acuity of 0.4 even at 53 years of age. Color vision was impaired in all patients (Table 2). Fundus findings were largely confined to the macular area, with very minimal RPE mottling early on and marked circumscribed atrophy, including the fovea and parafoveal area, in the more severe cases (Figs. 3A, 3B). These atrophic changes also were evident on FAF imaging, ranging from small hypofluorescent spots in milder stages of disease to large, well demarcated hypofluorescent areas surrounded by a hyperfluorescent ring in an advanced case. The OCT imaging similarly reflected the degree of macular RPE and photoreceptor involvement, with mild thinning of the photoreceptor layer in early phases, hypodense “cavitations” of the photoreceptor inner segment-outer segment complex in the foveal area in some cases (Fig. 3A), and up to complete loss of the photoreceptor layer and atrophy with choroidal backscatter in severe cases (Fig. 3B). The peripheral retina was largely within normal limits, except for changes associated with high myopia. Visual function testing corroborated the presence of a cone-dominated disease, with marked macular involvement. Full-field ERG testing invariably showed reduced cone amplitudes, often with prolonged implicit time (Table 2). Interestingly, in family MOL0508 with the novel c.2521G>A (p.E841K) mutation, cone 30Hz flicker amplitudes were reduced by only 25% to 50% of lower limit of normal, with implicit times at the upper limit of normal. Rod function was preserved in the majority of cases, mildly reduced in one and moderately reduced in the most severely affected patient (MOL0248 I:1). Electrooculography (EOG) testing was performed in 5 patients, with the Arden ratio being within normal limits in four of them. Patient MOL0430 I:1 with the novel c.2538G>C (p.K846N) variant had a reduced ratio of 170% in the RE and 133% in the LE at the age of 32 years (lower limit of normal 185%). Perimetry and microperimetry testing showed central partial and/or absolute scotomas (Figs. 3A, 3B), with preserved peripheral fields.

Recessive Families

In the recessive families MOL0056 and MOL0858, previously published genetic variants were identified in the *CDHR1* and *C8orf37* genes (Table 1). Whole exome sequencing analysis of one unaffected and 3 affected members of the consanguineous family MOL0056 (see Fig. 1B) revealed a known mutation, c.1381C>T (p.Q461*),²⁰ in exon 13 of the *CDHR1* gene. The three family members examined at the ages of 8, 12, and 20 (Table 2) showed widespread and progressive retinal degeneration. Mild to moderately reduced photopic and scotopic ERG responses were observed in the 8-year-old patient, severely reduced responses in the 12-year-old, and no detectable recordings in the 20-year-old individual. Goldmann visual fields, color vision impairment, and fundus findings likewise revealed increasing disease severity with age among

FIGURE 1. Pedigree structures of studied families. Family identifiers, gene names, and identified coding changes are shown. Segregation in the families is indicated as: M/M, homozygous for mutation; M/+, heterozygous; +/+ homozygous for wild type allele. *Filled symbols* represent affected individuals, *open symbols* represent unaffected individuals, a *small circle* signifies still birth. *Arrows* indicate index cases. Consanguineous marriages are represented by double lines. (A) Dominant families with *GUCY2D* mutations. (B) Recessive families with *CDHR1* and *C8orf37* mutations. (C) Pedigree of an unsolved family.

the two brothers (MOL0056 II:5 and II:6). Interestingly, although rod involvement per ERG seemed to exceed cone involvement, all patients noted a preference for dim light conditions and more difficulty in transition from dark to light than vice versa. Fundus autofluorescence findings suggest early macular involvement with hyperfluorescence in the foveal region already apparent in the 8-year-old patient (not shown).

In family MOL0858, previous whole genome SNP genotyping analysis of the index case (II:1) and his affected sister (II:2) revealed six large segregating regions of homozygosity (chr2, 8M-15M and 224M-228M; chr8, 76M-103M; chr11, 110M-118M;

chr16, 53M-59M; chr21, 23M-27M). Combining the homozygosity mapping data with WES analysis of the two affected and one unaffected member of the family (Fig. 1B) permitted us to identify a missense mutation, c.529C>T (p.R177W), in exon 6 of the *C8orf37* gene, which has been previously reported.²¹ Clinically, the two siblings manifested a CRD phenotype, with photophobia, marked color vision impairment, and cone-rod involvement in the ERG of the younger brother in whom responses still were detectable at 20 and 22 years of age (Table 2). Our findings are concordant with those reported for some of the patients with *C8orf37*-associated retinal disease.^{21,22}

TABLE 1. Genetic Variants Identified in Israeli Families With Primary Cone Involvement

Family Identifier	Clinical Diagnosis	Presumed Mode of Inheritance	Origin/Ethnic Group	Causative Gene (Exon)	Location of Nucleotide Change (Protein)*	Reference
MOL0490	CD/CRD	AD	Turkish Jewish	<i>GUCY2D</i> (13)	c.2512C>T (p.R838C) heterozygous	Kelsell et al. ¹⁷
MOL0859	CD	AD	Ashkenazi Jewish	<i>GUCY2D</i> (13)	c.2513G>A (p.R838H) heterozygous	Weigell-Weber et al. ¹⁸
MOL1103	CD/CRD	AD	Arab Muslim	<i>GUCY2D</i> (13)	c.2512C>T (p.R838C) heterozygous	Kelsell et al. ¹⁷
MOL0083	CD	AD	Ashkenazi Jewish	<i>GUCY2D</i> (13)	c.2513G>A (p.R838H) heterozygous	Weigell-Weber et al. ¹⁸
MOL0248	CRD	Isolate	Ashkenazi Jewish	<i>GUCY2D</i> (13)	c.2513G>A (p.R838H) heterozygous	Weigell-Weber et al. ¹⁸
MOL0430	CRD	Isolate	North African Jewish	<i>GUCY2D</i> (13)	c.2538G>C (p.K846N) heterozygous	Novel
MOL0508	Maculopathy + CD	AD with reduced penetrance	Ashkenazi Jewish	<i>GUCY2D</i> (13)	c.2521G>A (p.E841K) heterozygous	Novel
MOL0056	RD	AR	Arab Muslim	<i>CDHR1</i> (13)	c.1381C>T (p.Q461*) homozygous	Duncan et al. ²⁰
MOL0858	CRD	AR	Arab Muslim	<i>C8orf37</i> (6)	c.529C>T (p.R177W) homozygous	Estrada-Cuzcano et al. ²¹

RD, retinal dystrophy.

* The nucleotide position of each genetic variant is based on the following GenBank cDNAs (accession number): *GUCY2D* (NM_000180), *CDHR1* (NM_001171971), *C8orf37* (NM_177965).

In Family MOL0048, two male siblings presented mild to moderate cone dysfunction. No consanguinity was reported for the parents, and the mode of inheritance is thus presumed to be either XL or AR (Fig. 1C). Candidate gene screening as well as detailed analysis of WES data from the two affected siblings and their unaffected father did not reveal a potential causative genetic defect. It should be noted that the possible involvement of *RPGR*, including the ORF15 exon, which also is associated with cone-dominated phenotypes, was ruled out since the two affected brothers inherited different maternal alleles in this region.

DISCUSSION

Attaining a molecular genetic diagnosis in patients with hereditary retinal degenerations is becoming the standard of care. Once the causative gene is identified, prenatal and presymptomatic diagnoses can be offered, and gene-specific therapies, if available, can be realized. We have ongoing efforts to map phenotype and genotype in Israeli patients presenting retinal degenerative disease (RDD), identify novel or known genetic defects, and discover correlations between specific ethnic origins and potentially founder mutations. A more rapid and efficient subpopulation-specific genetic exploration already has led to application of gene augmentation therapy in patients of North-African Jewish descent with LCA caused by a founder mutation in the *RPE65* gene.³ Recent advent of techniques for high throughput sequencing such as WES and decreasing costs now allow us to pursue the goal of genetic diagnosis to an even greater extent, and this was applied in families with retinal degeneration characterized by predominant cone involvement, a phenotype that thus far has not been extensively studied in the Israeli population. In the present study, initial WES analysis followed by targeted mutation screening revealed *GUCY2D* gene mutations as a major cause of cone disease in the Israeli population.

To date, at least 10 genes associated with AD CD and CRD have been identified (RetNet), and mutations in *GUCY2D* were shown to be a major cause of AD cone disease in different ethnic groups worldwide,²³ with CD as the predominant phenotype.²⁴ A recent study conducted on a cohort of 52 patients and families with AD CD/CRD from Europe and the United States revealed 12 patients (23%) with mutations in the *GUCY2D* gene.²³ To our knowledge, this is the first report associating *GUCY2D* variants with AD cone phenotypes in the Israeli population, and the incidence (5 of 26 AD families, 19%) is similar to that observed in the aforementioned study. This finding is in contrast to what has been reported for AR rod-dominated retinal disease (RP and LCA) in Israel, in which causative genes and mutations largely differ from those reported in other populations.^{2-4,6}

The *GUCY2D* gene encodes a retina-specific guanylate cyclase predominantly expressed in photoreceptors, with the protein primarily localizing to the cone outer segments.²⁵ The *GUCY2D* gene has a key role in the phototransduction cascade by catalyzing the conversion of guanosine 5'-triphosphate (GTP) to cyclic guanosine monophosphate (cGMP) in a calcium-sensitive manner.²⁶ The increase of intracellular calcium concentration inhibits while a decrease stimulates cGMP synthesis. The *GUCY2D* gene contains 5 functional domains: an extracellular domain, a hydrophobic transmembrane domain, a kinase-like domain, a dimerization domain and a catalytic domain²⁷ (Fig. 2A). All four genetic variants identified in the present study are located within the dimerization domain of the protein and affect evolutionarily conserved amino acid residues. Codon 838 of the protein is altered by mutations in 5 families, and the two novel mutations affect neighboring positions, 841

FIGURE 2. Schematic representation of *GUCY2D* gene and protein structure, interspecies conservation of affected amino acid residues and chromatograms of the two novel variants. **(A)** The *GUCY2D* gene is composed of 20 exons that encode 5 protein domains: an extracellular domain, a transmembrane domain, a kinase-like domain, a dimerization domain, and a catalytic domain. Exons are color coded according to the respective encoded protein domains. All four genetic variants identified (*red*, novel; *black*, previously reported) are located in exon 13 of the gene and are predicted to affect the dimerization properties of the resulting protein monomers. **(B)** Interspecies conservation was generated using GenBank protein sequences (accession number) from human (NP_000171.1), chimpanzee (XP_003315414.1), rat (NP_077356.1), mouse (NP_032218.2), bovine (NP_776973.2), horse (XP_005597817.1), dog (NP_001003207.1), frog (XP_002942678.2), and zebrafish (NP_001103165.1). Amino acid residues affected by novel (*red frames*) and known (*black frame*) genetic variants are highly conserved. **(C)** Chromatograms showing the novel c.2521G>A and c.2538G>C heterozygous changes (*top*), and wild type allele (*bottom*) in affected and unaffected individuals (*arrows*).

and 846 in two other families. Amino acid position 838 is thought to be particularly prone to mutational changes due to the high number of cases observed in various ethnic backgrounds and is referred to as a mutation hotspot.^{13,18,23,28–31} Functional analysis of the mutant retinal guanylate cyclase at position 838 revealed a change in affinity toward regulation by calcium-bound inhibiting and calcium-free activating forms of the guanylate cyclase activating protein.²⁶ The disease is thought to result from an imbalance of cGMP and calcium in the photoreceptors. Heterozygous mutations in nearby residues also are associated with AD cone related phenotypes, including a heterozygous triple missense change (c.2540_2542delinsTCC, p.Q847_K848delinsLQ) affecting amino acid 847 and 848³² as well as a c.2545A>G transition that results in a threonine to alanine substitution at position 849.³³ Taken together, our results further highlight the importance of codon 838 and neighboring residues for normal function of the protein.

In addition to AD CD/CRD, mutations in the *GUCY2D* gene are associated with LCA and RP inherited in an autosomal recessive mode (Supplementary Table S2). Extensive literature on *GUCY2D* mutations prompted us to examine possible genotype-phenotype correlation. We note that all reported *GUCY2D* mutations in AD CD/CRD patients (except P575³⁴) are detected in the dimerization domain of the protein at or near residue R838. In contrast, the majority of LCA and RP patients carried homozygous *GUCY2D* mutations (except a heterozygous frame-shift mutation in one RP family³⁵) that were spread throughout the protein (Supplementary Table S2). We found a single report of dominant Central Areolar Choroidal Dystrophy with a *GUCY2D* mutation.³⁶ The role of some of the reported variants in causing disease is somewhat uncertain.^{36–39}

Our WES analysis of six families identified mutations in five of them, a relatively high detection rate that most probably is due to chance in the small number of families studied. The analysis of larger cohorts is required to ascertain the rate of mutation detection in RDDs by WES. Two of the AR families revealed causative mutations in previously reported genes. In one family, the c.1381C>T (p.Q461*) nonsense mutation was identified in the *CDHR1* gene, which encodes a photoreceptor specific cadherin that localizes to the base of rod and cone outer segments (OS) and is essential for OS integrity and photoreceptor survival.⁴⁰ The *CDHR1* protein is composed of a signal peptide, six cadherin domains, a transmembrane, and a cytoplasmic domain.⁴¹ Position 461 is located in the fourth cadherin domain of the protein and the p.Q461* mutation is predicted to either cause a premature truncation and a loss of 397 C-terminal amino acids²⁰ or to be subjected to nonsense-mediated mRNA decay and produce no protein. Mutations in *CDHR1* have been reported to cause AR-RP and AR-CRD in patients of Middle Eastern, South Asian, as well as Faroese descent.^{41–44} The clinical findings in the three affected patients reported here are similar to those described by Ba-Abaad et al.⁴⁴ in other young patients with *CDHR1* disease, in that rather severe and rapid retinal degeneration, as assessed by ERG, is accompanied by relatively subtle funduscopic changes, at least in the early stages. Interestingly, among the few families reported with *CDHR1* mutations, four are of Middle Eastern origin (as noted in prior studies^{20,41,43} and the family presented here). In the second AR family, the causative c.529C>T (p.R177W) mutation was identified in the *C8orf37* gene, which encodes a protein that localizes to the base of the photoreceptor connecting cilium.²¹ The *C8orf37* gene was reported previously to cause AR-RP and AR-CRD with a total of

TABLE 2. Clinical Findings in Patients With Disease-Causing Genetic Variants Identified in This Report

Patient No.	Clinical Diagnosis	Gene, Mutation	Visual Acuity (Age*)	Refraction (Age*)	Cone 30 Hz Flicker ERG μ V/IT (Age*)	DA Rod Response, b-Wave, μ V	DA Mixed Response a/b Wave, μ V	Comments / Observations
MO10490 III:1	CD/CRD	<i>GUCY2D</i> p.R838C	RE FC 1m LE 0.2 (32)	RE -10 LE -1 (32)	25 / 36 (32)	222	180 / 264	Anisometropia with RE, high myopia, and amblyopia; maculopathy with atrophy and RPE changes, periphery WNL; EOG: WNL; D-15: multiple scotopic lines.
MO10859 II:1	CD	<i>GUCY2D</i> p.R838H	0.4 (43, 53)	Plano	18 / 41 (52)	328	199 / 350	Photophobia; mild macular RPE changes, periphery WNL; EOG: WNL; D-15: scotopic lines.
MO11103 II:1	CD/CRD	<i>GUCY2D</i> p.R838C	0.3 (9, 12, 14)	-14 (12, 14)	Trace response (9)	241	167 / 260	Macula preserved, myopic changes; preserved color vision (at age 9).
MO11103 I:2	CD/CRD	<i>GUCY2D</i> p.R838C	0.15 (42)	High myopia	ND			Minimal macular changes.
MO11103 II:3	CD/CRD	<i>GUCY2D</i> p.R838C	0.5 (4.5)	-14 (3)	24 / 34 (3)		WNL (short protocol)	Tilted disks, poor foveal reflex.
MO10083 III:1	CD	<i>GUCY2D</i> p.R838H	RE 0.1 LE 0.7 (5)	-2 (5)	Trace response (35)	265	216 / 406	EOG: WNL; D-15 Scotopic lines.
MO10248 I:1	CRD	<i>GUCY2D</i> p.R838H	RE 0.05 LE 0.12 (59)	High myopia	Severely reduced (43) 8 / 39 (59)	113 (43) 119 (59)	88 / 134 (43) 112 / 112 (59)	Photophobia; Myopic changes, pale optic disks, macular atrophy R > L, periphery WNL; D-15: could not do.
MO10430 I:1	CRD	<i>GUCY2D</i> p.K846N	0.4 (16) 0.2 (23, 29, 30, 31)	-10 (25)	21 / 39 (29)	176	174 / 241	Photophobia; very mild RPE dropout in maculas (23); macular RPE changes L>R, periphery WNL (32); EOG: reduced; D-15: multiple nonspecific axes.
MO10508 III:1	Maculopathy + CD	<i>GUCY2D</i> p.E841K	0.3 (25)	-9 (25)	45 / 33 (25)	298	217 / 390	Myopic changes, a few yellow spots in the macular area; FA: small hyperfluorescent spots with no leakage; D-15: tritanopia.
MO10508 II:2	Maculopathy + CD	Affected mother of 508-1; DNA not available	0.25 (47)	-9 (47)	31 / 32 (47)	253	278 / 429	Mild maculopathy; EOG: WNL.
MO10056 I:2	RD	<i>CDHR1</i> p.Q461*	RE 0.05 LE 0.7 (20)	-2	Extinct (20)	Extinct	Extinct	Nystagmus; S&P changes, attenuated vessels, abnormal foveal color. EOG: Arden ratio 100%.

TABLE 2. Continued

Patient No.	Clinical Diagnosis	Gene, Mutation	Visual Acuity (Age ^a)	Refraction (Age ^a)	Cone 30 Hz Flicker ERG μ V/IT (Age ^a)	DA Rod Response, b-Wave, μ V	DA Mixed Response a/b Wave, μ V	Comments / Observations
MOI0056 II:5	RD	<i>CDHR1</i> p.Q461*	0.5 (13)	0	24 / 37 (12)	Extinct	44 / 97	S&P changes with mid-peripheral BSP; D-15; Tritanopia; Goldmann VFs: constricted to ~15°. 20° of fixation with VI4e target in BE. FAF: hyperfluorescence in foveal area.
MOI0056 II:6	RD	<i>CDHR1</i> p.Q461*	0.7 (8)	0	54 / 37 (8)	122	57 / 223	S&P changes; D-15: WNL; Goldmann VFs: constricted to ~40° of fixation with VI4e target in BE. FAF: hyperfluorescence in foveal area.
MOI0858 II:1	CRD	<i>C8orf37</i> p.R177W	0.5 (20)	High myopia	Extinct (20, 22)	88 (20) 74 (22)	70 / 118 (20) 28 / 91 (22)	Mild optic disc pallor and peripapillary atrophy, attenuated vessels, no pigmentary changes at age 20; D-15: multiple axes. OCT: marked thinning of photoreceptor layer in macula.
MOI0858 II:2	CRD	<i>C8orf37</i> p.R177W	FC 0.5 m (32)	−12 (33)	Extinct (33)	Extinct	Extinct	Photophobia; maculopathy, paravascular BSP, attenuated vessels with few ghost vessels in the periphery.

Data represent average between both eyes; when eyes differ, separate results are provided. Best corrected visual acuity given as ratio; when below 0.05, finger counting (FC) in meters is provided. Full-field ERG results include the following details: light-adapted cone flicker amplitude (normal 60–144 μ V) and implicit time (IT, in ms, normal 27–33 ms); dark-adapted rod response b-wave amplitude (normal range 200–500 μ V); and dark-adapted mixed cone–rod a- and b-wave amplitudes (normal a-wave 90–350 μ V, normal b-wave 380–630 μ V). In children, a short protocol with brief dark adaptation is performed under sedation. Cone responses are recorded as in adults. Refraction is given in diopters. RE, right eye; LE, left eye; BE, both eyes; EOG, electrooculography (normal Arden ratio > 185%); D-15, Farnsworth D-15 color test; VFs, visual fields; S&P, salt and pepper changes; BSP, bone-spicule-like pigmentation; ND, not done; WNL, within normal limits.

^a Age of testing is provided throughout the Table in parentheses. Clinical comments relate to age at which ERG was performed, unless otherwise stated.

FIGURE 3. Ocular findings in *GUCY2D*-associated retinal degeneration. (A) Retinal imaging and function in patient MOL0430 I:1, with relatively mild manifestation of disease. The CF photo and fluorescein angiography (FA) performed at age 24 reveal localized macular RPE changes with staining, which increased by age 30. A FAF study at age 30 shows small areas of atrophy in the foveal area and hypodense "foveal cavitation" is present on OCT (arrow). Static perimetry testing documents central scotomas in both eyes. LE, left eye; RE, right eye. (B) Ocular findings in patient MOL0248 I:1, manifesting severe disease at the age of 59. Multicolor, FAF and infrared (IR) imaging of the left eye show central macular atrophy, with loss of the photoreceptor layer in the foveal area on OCT (arrow). Symmetry between the left and right eyes is exemplified by the FAF images (note dark hypofluorescent area and surrounding hypofluorescent ring). Microperimetry examination of the right eye reveals an absolute central scotoma and reduced sensitivity beyond it, with poor fixation. (C) Microperimetry and imaging in a 32-year-old normal are shown for comparison.

five homozygous^{21,45} and one compound heterozygous mutation.⁴⁶

Our studies further support the use of WES in searching for genetic cause of disease, both for identification in specific families, but also as a source of information that can direct further screening by simpler methods in associated populations.^{47,48} Whole exome sequencing has been highly successful in discovering novel genes for retinal diseases,^{49–52} and can be efficient even in isolated cases or in families where only one or few samples are available for analysis.⁵³ In addition, WES allows genome-wide evaluation of modifier variants, the added

effect of which could potentially explain the variability in phenotype and disease progression observed among members of the same family. The continuous decrease in costs now makes this valuable tool increasingly lucrative, and even suitable for relatively large-scale molecular genetic screens. However, limitations do exist, as exemplified by the family in which the genetic cause could not be determined even after performing WES. This may be due to insufficient coverage of specific exons, occurrence of genomic alterations/deletions that are difficult to identify, mutations within intronic regions,

and interactions between two or more genes that may cause disease.

In summary, exome sequencing has assisted us in identifying causative genes and mutations in a number of retinal degeneration families with primary cone disease. The *GUCY2D* gene has now emerged as a major cause of AD cone-dominated disease in the Israeli population, but it explains less than 20% of cases even within this limited phenotype. A comprehensive analysis of the Israeli RDD cohort is currently ongoing to identify genetic defects by updated screening panels for newly recruited families (including the *GUCY2D* mutational hotspot in AD families) as well as by next generation sequencing techniques.

Acknowledgments

The authors thank Matthew Brooks for technical advice, and Alexey Obolensky, Shelly Stika, and Inbar Erdinest for assistance in clinical evaluation of the patients.

Supported by the United States-Israel Binational Science Foundation (Grant 2011202; DS, AS), the Yedidut Research Grant (EB), and the Intramural Research Program of the National Eye Institute, National Institutes of Health (AS).

Disclosure: **C.H. Lazar**, None; **M. Mutsuddi**, None; **A. Kimchi**, None; **L. Zelinger**, None; **L. Mizrahi-Meissonnier**, None; **D. Marks-Ohana**, None; **A. Boleda**, None; **R. Ratnapriya**, None; **D. Sharon**, None; **A. Swaroop**, None; **E. Banin**, None

References

- Zlotogora J. The molecular basis of autosomal recessive diseases among the Arabs and Druze in Israel. *Hum Genet.* 2010;128:473-479.
- Auslender N, Sharon D, Abbasi AH, Garzozzi HJ, Banin E, Ben-Yosef T. A common founder mutation of CERKL underlies autosomal recessive retinal degeneration with early macular involvement among Yemenite Jews. *Invest Ophthalmol Vis Sci.* 2007;48:5431-5438.
- Banin E, Bandah-Rozenfeld D, Obolensky A, et al. Molecular anthropology meets genetic medicine to treat blindness in the North African Jewish population: human gene therapy initiated in Israel. *Hum Gene Ther.* 2010;21:1749-1757.
- Zelinger L, Banin E, Obolensky A, et al. A missense mutation in DHDDS, encoding dehydrosulfolipyl diphosphate synthase, is associated with autosomal-recessive retinitis pigmentosa in Ashkenazi Jews. *Am J Hum Genet.* 2011;88:207-215.
- Beit-Ya'acov A, Mizrahi-Meissonnier L, Obolensky A, et al. Homozygosity for a novel ABCA4 founder splicing mutation is associated with progressive and severe Stargardt-like disease. *Invest Ophthalmol Vis Sci.* 2007;48:4308-4314.
- Bandah-Rozenfeld D, Mizrahi-Meissonnier L, Farhy C, et al. Homozygosity mapping reveals null mutations in FAM161A as a cause of autosomal-recessive retinitis pigmentosa. *Am J Hum Genet.* 2010;87:382-391.
- Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet.* 2006;368:1795-1809.
- Venturini G, Di Gioia SA, Harper S, Weigel-DiFranco C, Rivolta C, Berson EL. Molecular genetics of FAM161A in North American patients with early-onset retinitis pigmentosa. *PLoS One.* 2014;9:e92479.
- Roosing S, Thiadens AA, Hoyng CB, Klaver CC, den Hollander AI, Cremers FP. Causes and consequences of inherited cone disorders. *Prog Retin Eye Res.* 2014;42:1-26.
- Michaelides M, Hardcastle AJ, Hunt DM, Moore AT. Progressive cone and cone-rod dystrophies: phenotypes and underlying molecular genetic basis. *Surv Ophthalmol.* 2006;51:232-258.
- Berger W, Kloeckener-Gruissem B, Neidhardt J. The molecular basis of human retinal and vitreoretinal diseases. *Prog Retin Eye Res.* 2010;29:335-375.
- Hamel CP. Cone rod dystrophies. *Orphanet J Rare Dis.* 2007;2:7.
- Kitiratschky VB, Wilke R, Renner AB, et al. Mutation analysis identifies GUCY2D as the major gene responsible for autosomal dominant progressive cone degeneration. *Invest Ophthalmol Vis Sci.* 2008;49:5015-5023.
- Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics.* 2009;25:2078-2079.
- Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* 2010;38:e164.
- Thorvaldsdottir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform.* 2013;14:178-192.
- Kellsell RE, Gregory-Evans K, Payne AM, et al. Mutations in the retinal guanylate cyclase (RETGC-1) gene in dominant cone-rod dystrophy. *Hum Mol Genet.* 1998;7:1179-1184.
- Weigell-Weber M, Fokstuen S, Torok B, Niemeyer G, Schinzel A, Hergersberg M. Codons 837 and 838 in the retinal guanylate cyclase gene on chromosome 17p: hot spots for mutations in autosomal dominant cone-rod dystrophy? *Arch Ophthalmol.* 2000;118:300.
- Zobor D, Zrenner E, Wissinger B, Kohl S, Jagle H. GUCY2D- or GUCA1A-related autosomal dominant cone-rod dystrophy: is there a phenotypic difference? *Retina.* 2014;34:1576-1587.
- Duncan JL, Roorda A, Navani M, et al. Identification of a novel mutation in the CDHR1 gene in a family with recessive retinal degeneration. *Arch Ophthalmol.* 2012;130:1301-1308.
- Estrada-Cuzcano A, Neveling K, Kohl S, et al. Mutations in C8orf37, encoding a ciliary protein, are associated with autosomal-recessive retinal dystrophies with early macular involvement. *Am J Hum Genet.* 2012;90:102-109.
- van Huet RA, Estrada-Cuzcano A, Banin E, et al. Clinical characteristics of rod and cone photoreceptor dystrophies in patients with mutations in the C8orf37 gene. *Invest Ophthalmol Vis Sci.* 2013;54:4683-4690.
- Kohl S, Kitiratschky V, Papke M, Schaich S, Sauer A, Wissinger B. Genes and mutations in autosomal dominant cone and cone-rod dystrophy. *Adv Exp Med Biol.* 2012;723:337-343.
- Hunt DM, Buch P, Michaelides M. Guanylate cyclases and associated activator proteins in retinal disease. *Mol Cell Biochem.* 2010;334:157-168.
- Liu X, Seno K, Nishizawa Y, et al. Ultrastructural localization of retinal guanylate cyclase in human and monkey retinas. *Exp Eye Res.* 1994;59:761-768.
- Peshenko IV, Moiseyev GP, Olshevskaya EV, Dizhoor AM. Factors that determine Ca²⁺ sensitivity of photoreceptor guanylyl cyclase. Kinetic analysis of the interaction between the Ca²⁺-bound and the Ca²⁺-free guanylyl cyclase activating proteins (GCAPs) and recombinant photoreceptor guanylyl cyclase 1 (RetGC-1). *Biochemistry.* 2004;43:13796-13804.
- Potter LR. Domain analysis of human transmembrane guanylyl cyclase receptors: implications for regulation. *Front Biosci.* 2005;10:1205-1220.
- Payne AM, Morris AG, Downes SM, et al. Clustering and frequency of mutations in the retinal guanylate cyclase (GUCY2D) gene in patients with dominant cone-rod dystrophies. *J Med Genet.* 2001;38:611-614.
- Ito S, Nakamura M, Ohnishi Y, Miyake Y. Autosomal dominant cone-rod dystrophy with R838H and R838C mutations in the GUCY2D gene in Japanese patients. *Jpn J Ophthalmol.* 2004;48:228-235.

30. Xiao X, Guo X, Jia X, Li S, Wang P, Zhang Q. A recurrent mutation in GUCY2D associated with autosomal dominant cone dystrophy in a Chinese family. *Mol Vis.* 2011;17:3271-3278.
31. Garcia-Hoyos M, Auz-Alexandre CL, Almoguera B, et al. Mutation analysis at codon 838 of the Guanylate Cyclase 2D gene in Spanish families with autosomal dominant cone, cone-rod, and macular dystrophies. *Mol Vis.* 2011;17:1103-1109.
32. Yoshida S, Yamaji Y, Yoshida A, et al. Novel triple missense mutations of GUCY2D gene in Japanese family with cone-rod dystrophy: possible use of genotyping microarray. *Mol Vis.* 2006;12:1558-1564.
33. Zhao X, Ren Y, Zhang X, Chen C, Dong B, Li Y. A novel GUCY2D mutation in a Chinese family with dominant cone dystrophy. *Mol Vis.* 2013;19:1039-1046.
34. Small KW, Silva-Garcia R, Udar N, Nguyen EV, Heckenlively JR. New mutation, P575L, in the GUCY2D gene in a family with autosomal dominant progressive cone degeneration. *Arch Ophthalmol.* 2008;126:397-403.
35. Booi JC, Florijn RJ, ten Brink JB, et al. Identification of mutations in the AIPL1, CRB1, GUCY2D, RPE65, and RPGRIP1 genes in patients with juvenile retinitis pigmentosa. *J Med Genet.* 2005;42:e67.
36. Hughes AE, Meng W, Lotery AJ, Bradley DT. A novel GUCY2D mutation, V933A, causes central areolar choroidal dystrophy. *Invest Ophthalmol Vis Sci.* 2012;53:4748-4753.
37. Perrault I, Rozet JM, Gerber S, et al. Spectrum of retGC1 mutations in Leber's congenital amaurosis. *Eur J Hum Genet.* 2000;8:578-582.
38. Hancin S, Perrault I, Gerber S, et al. Leber congenital amaurosis: comprehensive survey of the genetic heterogeneity, refinement of the clinical definition, and genotype-phenotype correlations as a strategy for molecular diagnosis. *Hum Mutat.* 2004;23:306-317.
39. Li L, Xiao X, Li S, et al. Detection of variants in 15 genes in 87 unrelated Chinese patients with Leber congenital amaurosis. *PLoS One.* 2011;6:e19458.
40. Rattner A, Smallwood PM, Williams J, et al. A photoreceptor-specific cadherin is essential for the structural integrity of the outer segment and for photoreceptor survival. *Neuron.* 2001;32:775-786.
41. Henderson RH, Li Z, Abd El Aziz MM, et al. Biallelic mutation of protocadherin-21 (PCDH21) causes retinal degeneration in humans. *Mol Vis.* 2010;16:46-52.
42. Ostergaard E, Batbayli M, Duno M, Vilhelmsen K, Rosenberg T. Mutations in PCDH21 cause autosomal recessive cone-rod dystrophy. *J Med Genet.* 2010;47:665-669.
43. Cohen B, Chervinsky E, Jabaly-Habib H, Shalev SA, Briscoe D, Ben-Yosef T. A novel splice site mutation of CDHR1 in a consanguineous Israeli Christian Arab family segregating autosomal recessive cone-rod dystrophy. *Mol Vis.* 2012;18:2915-2921.
44. Ba-Abbad R, Sergouniotis PI, Plagnol V, et al. Clinical characteristics of early retinal disease due to CDHR1 mutation. *Mol Vis.* 2013;19:2250-2259.
45. Jinda W, Taylor TD, Suzuki Y, et al. Whole exome sequencing in Thai patients with retinitis pigmentosa reveals novel mutations in six genes. *Invest Ophthalmol Vis Sci.* 2014;55:2259-2268.
46. Katagiri S, Hayashi T, Yoshitake K, et al. Novel C8orf37 mutations in patients with early-onset retinal dystrophy, macular atrophy, cataracts, and high myopia. *Ophthalmic Genet.* 2014;1-8.
47. Collin RW, Nikopoulos K, Dona M, et al. ZNF408 is mutated in familial exudative vitreoretinopathy and is crucial for the development of zebrafish retinal vasculature. *Proc Natl Acad Sci U S A.* 2013;110:9856-9861.
48. El Shamieh S, Neuville M, Terray A, et al. Whole-exome sequencing identifies KIZ as a ciliary gene associated with autosomal-recessive rod-cone dystrophy. *Am J Hum Genet.* 2014;94:625-633.
49. Sergouniotis PI, Chakarova C, Murphy C, et al. Biallelic variants in TLL5, encoding a tubulin glutamylase, cause retinal dystrophy. *Am J Hum Genet.* 2014;94:760-769.
50. Roosing S, Lamers IJ, de Vrieze E, et al. Disruption of the basal body protein POC1B results in autosomal-recessive cone-rod dystrophy. *Am J Hum Genet.* 2014;95:131-142.
51. Davidson AE, Schwarz N, Zelinger L, et al. Mutations in ARL2BP, encoding ADP-ribosylation-factor-like 2 binding protein, cause autosomal-recessive retinitis pigmentosa. *Am J Hum Genet.* 2013;93:321-329.
52. Bujakowska KM, Zhang Q, Siemiatkowska AM, et al. Mutations in IFT172 cause isolated retinal degeneration and Bardet-Biedl syndrome. *Hum Mol Genet.* 2015;24:230-242.
53. Corton M, Nishiguchi KM, Avila-Fernandez A, et al. Exome sequencing of index patients with retinal dystrophies as a tool for molecular diagnosis. *PLoS One.* 2013;8:e65574.