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RETINAL DISORDERS

# Clinical-genetic findings in a group of subjects with macular dystrophies due to mutations in rare inherited retinopathy genes

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## Abstract

**Purpose** To describe the results of clinical and molecular analyses in a group of patients suffering from inherited macular dystrophies, in which next-generation sequencing (NGS) efficiently detected rare causative mutations.

**Methods** A total of eight unrelated Mexican subjects with a clinical and multimodal imaging diagnosis of macular dystrophy were included. Visual assessment methods included best corrected visual acuity, color fundus photography, Goldmann visual field tests, kinetic perimetry, dark/light adapted chromatic perimetry, full-field electroretinography, autofluorescence imaging, and spectral domain-optical coherence tomography imaging. Genetic screening was performed by means of whole exome sequencing with subsequent Sanger sequencing validation of causal variants.

**Results** All patients exhibited a predominantly macular or cone-dominant disease. Patients' ages ranged from 12 to 60 years. Three cases had mutations in genes associated with autosomal dominant inheritance (*UNC119* and *PRPH2*) while the remaining five cases had mutations in genes associated with autosomal recessive inheritance (*CNGA3*, *POC1B*, *BEST1*, *CYP2U1*, and *PROM1*). Of the total of 11 different pathogenic alleles identified, three were previously unreported disease-causing variants.

**Conclusions** Macular dystrophies can be caused by defects in genes that are not routinely analyzed or not included in NGS gene panels. In this group of patients, whole exome sequencing efficiently detected rare genetic causes of hereditary maculopathies, and our findings contribute to expanding the current knowledge of the clinical and mutational spectrum associated with these disorders.

### Key messages

- Inherited maculopathies are a group of clinically and genetically heterogeneous diseases associated with mutations in nearly 40 different genes.
- Exome sequencing efficiently revealed rare genetic causes of hereditary maculopathies in a group of Mexican patients described here.
- A total of 11 different pathogenic alleles were identified, including three previously unreported disease-causing variants, thus expanding the knowledge of the molecular basis of hereditary maculopathies.

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**Keywords** Retinal dystrophy · Macular dystrophy · Exome sequencing · *UNC11* gene · *POC1B* gene

## Introduction

Inherited retinal dystrophies, the main cause of incurable familial blindness in the Western world, are a group of phenotypically and genetically heterogeneous conditions characterized by progressive degeneration of photoreceptors and retinal pigment epithelial cells [1]. A large number of inherited retinal dystrophies primarily affect the macula, the part of the retina specialized for central vision and advanced visual function. The term hereditary macular dystrophies (HMDs) describes a group of retinopathies characterized by symmetrical and progressive central vision loss due to degeneration of the macula and the underlying retinal pigment epithelium (RPE), with preferential damage of cones [2]. While HMDs can exhibit great phenotypic variability, they are characterized by funduscopic changes involving the macula and RPE, clinically significant loss of central vision, and gradual loss of acuity, color vision, and contrast sensitivity [3]. Stargardt disease (STGD) is the most prevalent HMD with an estimated incidence of one in 10,000 [4, 5]. The typical presentation is an autosomal recessive form with onset between 7 and 12 years of age [6]. Classical STGD (OMIM #248200) is caused by biallelic mutations in the *ABCA4* gene [7]. Another prevalent HRD is Best vitelliform macular dystrophy (Best disease, OMIM #153700), which is considered the second most common form of juvenile macular degeneration [8]. Best disease is caused by dominant mutations in the *BEST1* gene [9].

According to RetNet (https://sph.uth.edu/retnet/sum-dis.htm, accessed in August 2021), there are currently more than 50 identified genes associated with inherited maculopathies and/or with retinopathies with degeneration predominantly of the cone photoreceptors. Thus, providing a molecular diagnosis in HMDs is challenging due to the large numbers of causative genes.

The continuously increasing use of next-generation sequencing (NGS) for genetic analysis of a growing number of patients with HMD has not only allowed many of them to receive a precise molecular diagnosis, but has also enabled the recognition of additional genes causing hereditary maculopathy such as *CLN3* [10], *CRB1* [11], *TLCD3B* [12], *PRDM13* [13], *MFSD8* [14], *FBN2* [15], and *DRAM2* [16], among others. NGS is rapidly becoming a practical first-tier test as the cost decreases and performance improves, and its growing application in clinical settings offers the opportunity to detect mutations underlying rare inherited maculopathies.

Here, we describe the results of the clinical and molecular analyses in a group of patients suffering from inherited macular dystrophies in which whole exome sequencing efficiently detected causative mutations in uncommon maculopathy genes.

## Material and methods

### Clinical assessment

The protocol was approved by Institutional Review Board of the Institute of Ophthalmology "Conde de Valenciana" in Mexico City. All procedures followed the tenets of the Helsinki Declaration and patients gave written permission for their inclusion in the study. Participating subjects underwent a complete eye examination including best corrected visual acuity (BCVA), color fundus photography, Goldmann visual field kinetic perimetry, dark/light adapted chromatic perimetry, full-field electroretinography (ERG), autofluorescence imaging (FAF), and spectral domain-optical coherence tomography (SD-OCT) imaging, as previously detailed [17]. Full-field ERG incorporated recommendations of the International Society for Clinical Electrophysiology of Vision (ISCEV) were followed [18]. OCT images were acquired using a spectral-domain system (Heidelberg Spectralis OCT, Heidelberg Engineering, Heidelberg, Germany) and horizontal and vertical line scans centered at the fovea were obtained.

### Exome sequencing

Genomic DNA was extracted from peripheral blood leukocytes from affected subjects using the QIAmp DNA Blood kit (Qiagen, Hilden, Germany). DNA quantification and purity were measured using a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Library preparation for exome sequencing was performed using the Agilent SureSelect Human All Exon V6 kit (Agilent Technologies, Santa Clara, CA, USA). DNA was fragmented and purified using an Agencourt AMPure XP kit (Beckman Coulter Genomics, Chaska, MN, USA). DNA fragment ends were repaired, and adaptor sequences were added to the 5′ and 3′ ends of all fragments. Subsequently, each library was purified, amplified, and hybridized to the SureSelect Human All Exon V6 probes. Index adaptors were ligated to the 5′ and 3′ ends of each sample. DNA fragments were amplified, and fragments from 250 to 350 bp were isolated. The quality of

the libraries was assessed through a Bioanalyzer 2100 (Agilent Technologies). Lastly, 100 bp paired-end sequencing was performed using a HiSeq NGS platform (Illumina, San Diego, CA, USA). The average target region coverage for the samples was 98.2% at ≥ 50×. Exome sequencing data was filtered using the Franklin platform (Genoox, Palo Alto, CA, USA) available at https://franklin.genoox.com/clinical-db/home. Designation of pathogenic or likely pathogenic variants was carried out according to the American College of Genetics and Genomics guidelines [19]. Variants of clinical significance were confirmed using Sanger sequencing. Copy number variant (CNV) analyses were also conducted using the Franklin platform algorithm *Rainbow*.

## Results

A total of eight unrelated Mexican subjects with a clinical and multimodal imaging diagnosis of macular dystrophy were included. Three individuals had a history of additional affected relatives while the remaining five were apparently sporadic cases. Patients' age ranged from 12 to 60 years and five of them were male. Table 1 summarizes the multimodal imaging and ERG test results for all eight patients. Supplementary Figs. 1 and 2 show ERG traces and visual fields for some of the patients. Three cases had mutations in genes associated with autosomal dominant inheritance (*UNC119* and *PRPH2*) while the remaining five cases had mutations in genes associated with autosomal recessive inheritance (*CNGA3*, *POC1B*, *BEST1*, *CYP2U1*, and *PROM1*). Of the total of 11 pathogenic alleles identified, three were previously unreported mutations. Table 2 summarizes the molecular genetic findings in the cohort. Sanger sequencing traces of the identified variants are shown in Supplementary Fig. 3.

### Patient #1

This is a 55-year-old male with a recent diagnosis of macular degeneration. He began noticing nyctalopia 3 years ago. His mother and a maternal aunt, both deceased, also had a macular degeneration diagnosis. He reported wearing glasses since the age of 10 and had a history of refractive surgery. At the present examination, his BCVA was 0.1 logMAR (0.8 Snellen decimal) in the right eye and 0.1 logMAR (0.8 Snellen decimal) in the left eye. No systemic anomalies were recorded. Fundus examination disclosed bilateral macular drusen-like deposits (Fig. 1A–F). Visual field examination and ERG led to the diagnosis cone-rod dystrophy. Exome sequencing in this patient revealed a previously unreported heterozygous c.601G > T (p.Glu201Ter) mutation in the *UNC119* gene.

### Patient #2

This patient is a 60-year-old male complaining of blurry vision since infancy, color vision deficiency starting at 15 years of age, and photophobia since the age of 30. No family history of visual diseases was reported and no systemic anomalies were observed at physical examination. His BCVA was 0.69 logMAR (0.1 Snellen decimal) in the right eye and 0.84 logMAR (0.14 Snellen decimal) in the left eye. On fundus examination he had macular hyperpigmentation, macular folds and retinal sheen, posterior staphyloma and scleral crescent in the right eye, and hypopigmented macular dots in the left eye (Fig. 1G–L).

NGS analysis in this subject revealed two heterozygous variants in the *CNGA3* gene: c.1541A > T (P.Asp514val) and c.1981C > A (p.Arg661Ser). Familial genetic analysis by Sanger sequencing demonstrated that both variants were in the *trans* configuration (i.e., biallelic mutations).

### Patient #3

This is a 31-year-old female complaining of photophobia since the age of 10, dyschromatopsia from the age of 18, and visual loss at the age of 22. She had no family history of similar ocular disease. Systemic features were identified and included mild hearing loss and hyposmia. She had a history of hypercholesterolemia, hypertriglyceridemia, and hyperprolactinemia with galactorrhea. She developed an idiopathic intracranial hypertension episode at the age of 31 years that required ventriculoperitoneal shunt placement. BCVA was 0.8 logMAR (0.16 Snellen decimal) in the right eye and 0.8 logMAR (0.16 Snellen decimal) in the left eye. Fundus examination showed a bilateral pale optic disk with normal cupping, a bilateral parafoveal ring of hyperpigmentation, and a peripapillary myelinated retinal nerve fiber layer in the left eye (Fig. 1M–R). Whole exome sequencing in this individual identified heterozygosity for the previously unreported c.144delG (p.Lys48AsnfsTer16) and for the previously reported c.101-3 T > G mutations in the *POC1B* gene. Sanger sequencing in available relatives confirmed that *POC1B* variants were in separate alleles (i.e., compound heterozygosity).

### Patient #4

A 12-year-old female patient born from a first-cousin marriage was evaluated at the age of 11 years because of blurry vision. BCVA was 0.5 logMAR (0.32 Snellen decimal) in the right eye and 0.3 logMAR (0.5 Snellen decimal) in the left eye. Fundus examination showed a hyperemic optic disk with normal cupping and parafoveal ring hyperpigmentation and hypopigmentation in the middle peripheral retina, bilaterally (Fig. 2A–F). A previously unreported homozygous

**Table 1** Clinical and multimodal imaging features of patients with inherited maculopathies

| Patient # | Gender/age (years) | Funduscopic findings | OCT | Kinetic Goldmann fields | Dark/light adapted chromatic perimetry | ERG |
|---|---|---|---|---|---|---|
| 1 | M/55 | Bilateral macular drusen-like deposits | Loss of RPE continuity in the foveolar area (OU); subretinal hyperreflective deposits involving the foveal center | V4e stimuli: absolute scotoma within normal parameters; 14E stimuli: generalized increase in the relative scotoma with inferior predominance | Scotopic: mean deficit of 4.14 dB ($p < 0.05$)<br>Photopic: mean deficit of 0.15 dB ($p < 0.05$) in the whole visual field | Normal scotopic and photopic responses |
| 2 | F/60 | Scattered central hypopigmented lesions with hypopigmentation in middle peripheral retina (OD); scattered hypopigmented lesions in the middle peripheral retina (OS) | Central foveal thinning associated with loss of hyperreflective bands and subretinal hyperreflective lesion (OD); preservation of outer retinal lamination; subretinal hyperreflective deposits (OS) | V4e stimuli: functional evaluation of retinal cones showed an absolute scotoma within normal parameters (OD)<br>14E stimuli: increase of the relative scotoma and a 5° cecocentral scotoma (OD) | Scotopic: mean deficit of 1.22 dB ($p < 0.05$) in all the visual field<br>Photopic: mean deficit of 0.05 dB ($p < 5$) in all the visual field | Normal scotopic and photopic responses |
| 3 | F/31 | Bilateral temporal optic disk pallor; peripapillary myelinated retinal nerve fiber layer (OS); parafoveal ring hyperpigmentation (OU) | Preservation of inner and outer retinal lamination (OU) | V4e stimuli: absolute scotoma within normal parameters; 14E stimuli: cecocentral scotoma < 5° | Scotopic: mean deficit of 1.4 dB ($p < 0.05$) in all the visual field; generalized decrease of sensitivity, predominantly on the peripheral retina<br>Photopic: a mean deficit of 16 dB ($p < 0.05$) in all the visual field (OD) | Normal scotopic responses; absent photopic responses |
| 4 | F/12 | Hyperemic optic disks, foveolar hypopigmented mottling; parafoveal ring hyperpigmentation and hypopigmentation in middle peripheral retina (OU) | Loss of RPE integrity with intraretinal hyporeflective lesions on the inner retina, subretinal fluid (OU) | V4e and 14E stimuli: normal parameters | Scotopic: mean deficit of 12.25 dB ($p < 0.05$), in all the visual field (OS); global sensitivity loss with predominance at nasal peripheral retina<br>Photopic: mean deficit of 3.28 dB ($p < 0.05$) in all the visual field (OD); a global loss of sensitivity with nasal predominance and complete loss of sensitivity 50° nasally to the foveal center | Normal scotopic and photopic responses |
| 5 | M/39 | Macular scar and hyperpigmentation surrounded by a hypopigmented ring (OU) | Thinning of foveal retinal area; loss of outer retinal lamination involving the foveal; hyperreflectivity subretinal lesion (OU) | V4e and 14E stimuli: normal parameters | Scotopic: mean deficit of 1.7 dB ($p < 0.05$) in all the visual field; mild sensitivity loss in the superior peripheral retina and major loss in meridian 5 at 10° in the inferior nasal retina<br>Photopic: mean deficit of 1.8 dB ($p < 0.05$), in all the visual field; global sensitivity loss with superior retina predominance | Normal scotopic and photopic responses |

**Table 1** (continued)

| Patient # | Gender/age (years) | Funduscopic findings | OCT | Kinetic Goldmann fields | Dark/light adapted chromatic perimetry | ERG |
|---|---|---|---|---|---|---|
| 6 | M/37 | Macular hypopigmentation and hyperpigmented spots with parafoveal confluence forming a ring (OU) | Thinning of neurosensorial retinal layers with loss of external hyperreflective bands of parafoveal retina and preservation of foveal external hyperreflective bands (OU) | Not done | Scotopic: mean deficit of 1.7 dB ($p < 0.05$) in all the visual field; generalized loss of sensitivity mainly at central area<br>Photopic: mean deficit of dB ($p < 0.05$), in all the visual field; complete sensitivity loss in 10° of central macula | Normal scotopic and photopic responses |
| 7 | M/42 | Macular retinochoroidal atrophy patches surrounded by discrete yellowish, round, and pisciform flecks and peripapillary atrophy (OU) | Loss of outer retinal lamination involving the foveal center (OU); hyperreflective lesion under the fovea (OD) | V4e stimuli: 30° reduction at temporal visual field and 20° reduction at inferior, nasal, and superior fields<br>14E stimuli: generalized increase of the relative scotoma with temporal predominance | Scotopic: mean deficit of 18 dB ($p < 0.05$) in all the visual field<br>Photopic: mean deficit of 9 dB ($p > 0.05$) in all the visual field | Scotopic: absence of peripheral rod response<br>Photopic: absence of peripheral cone response |
| 8 | F/47 | Macular scar (OS); macular atrophy and drusen (OU) | Paracentral loos of outer nuclear layer and outer retinal lamination (OD); thinning of foveal center associated with loss of outer nuclear layer and outer retinal lamination (OS) | V4e and 14E stimuli: normal parameters (OD)<br>V4e: normal (OD); 14E stimuli: centrocecal scotoma minor than 10° (OS) | Scotopic: mean deficit of dB ($p < 0.05$) in all the visual field; it also exhibits a complete loss of sensitivity in MII of peripheral retina<br>Photopic: mean deficit of 1.8 dB ($p < 0.05$), in all the visual field | Normal scotopic and photopic responses |

**Table 2** Molecular findings in the cohort of macular dystrophy patients

| Patient # | Gene | Zygosity | Disease [OMIM]/ inheritance | c.DNA change | Protein change | ACMG* classification (criteria) | Previously reported (ref.) |
|---|---|---|---|---|---|---|---|
| 1 | *UNC119* | Heterozygous | Cone-rod dystrophy [N/A]/AD | c.601G > T | p.Glu201Ter | Likely pathogenic (PVS1, PM2, PP3) | No |
| 2 | *CNGA3* | Compound heterozygous | Achromatopsia 2 [#216900]/AR | c.1541A > T<br>c.1981C > A | p.Asp514Val<br>p.Arg661Ser | Likely pathogenic (PM1, PM2, PP2, PP3)<br>Likely pathogenic (PM2, PP2, PP3, PP5) | Yes [20]<br>Yes [21] |
| 3 | *POC1B* | Compound heterozygous | Cone-rod dystrophy 20 [#615973]/AR | c.144delG (allele 1)<br>c.101-3 T > G (allele 2) | p.Lys48AsnfsTer16<br>Splicing | Pathogenic (PVS1, PM2, PP5)<br>Likely pathogenic (PM2, PP3, PP5) | No<br>Yes [22] |
| 4 | *BEST1* | Homozygous | Bestrophinopathy, recessive [#611809]/ AR | c.70 T > C | p.Trp24Arg | Likely pathogenic (PM1, PM2, PM5, PP3, PP2) | No |
| 5 | *CYP2U1* | Homozygous | Spastic paraplegia 56 [#615030]/AR | c.1168C > T | p.Arg390Ter | Pathogenic (PVS1, PM2, PP5) | Yes [23] |
| 6 | *PRPH2* | Heterozygous | Macular dystrophy [#169150; #608161]/ AD | c.749G > A | p.Cys250Tyr | Likely pathogenic (PM1, PM2, PM5, PP2, PP3, PP5) | Yes [24] |
| 7 | *PROM1* | Compound heterozygous | Macular dystrophy 2 [#608051]; cone-rod dystrophy 12 [#612657]/AD, AR | c.1354dupT (allele 1)<br>c.1984-1G > T (allele 2) | p.Tyr452fs<br>Splicing | Pathogenic (PVS1, PS4, PM2, PP5)<br>Pathogenic (PVS1, PM2, PP5) | Yes [25]<br>Yes [26] |
| 8 | *PRPH2* | Heterozygous | Macular dystrophy [#169150; #608161]/ AD | c.424C > T | p.Arg142Trp | Pathogenic (PM1, PM2, PM5, PP2, PP5) | Yes [27] |

*AD*, autosomal dominant; *AR*, autosomal recessive; *American College of Medical Genetics and Genomics

ACMG criteria:

Very strong (PVS):

1. Null variant (nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease

Strong (PS):

1. Same amino acid change as a previously established pathogenic variant regardless of nucleotide change
2. De novo (both maternity and paternity confirmed) in a patient with the disease and no family history
3. Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product
4. The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls

Moderate (PM):

1. Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation
2. Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
3. For recessive disorders, detected in trans with a pathogenic variant
4. Protein length changes as a result of in-frame deletions/insertions in a non-repeat region or stop-loss variants
5. Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before
6. Assumed de novo, but without confirmation of paternity and maternity

Supporting (PP):

1. Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease
2. Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease
3. Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)
4. Patient's phenotype or family history is highly specific for a disease with a single genetic etiology
5. Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

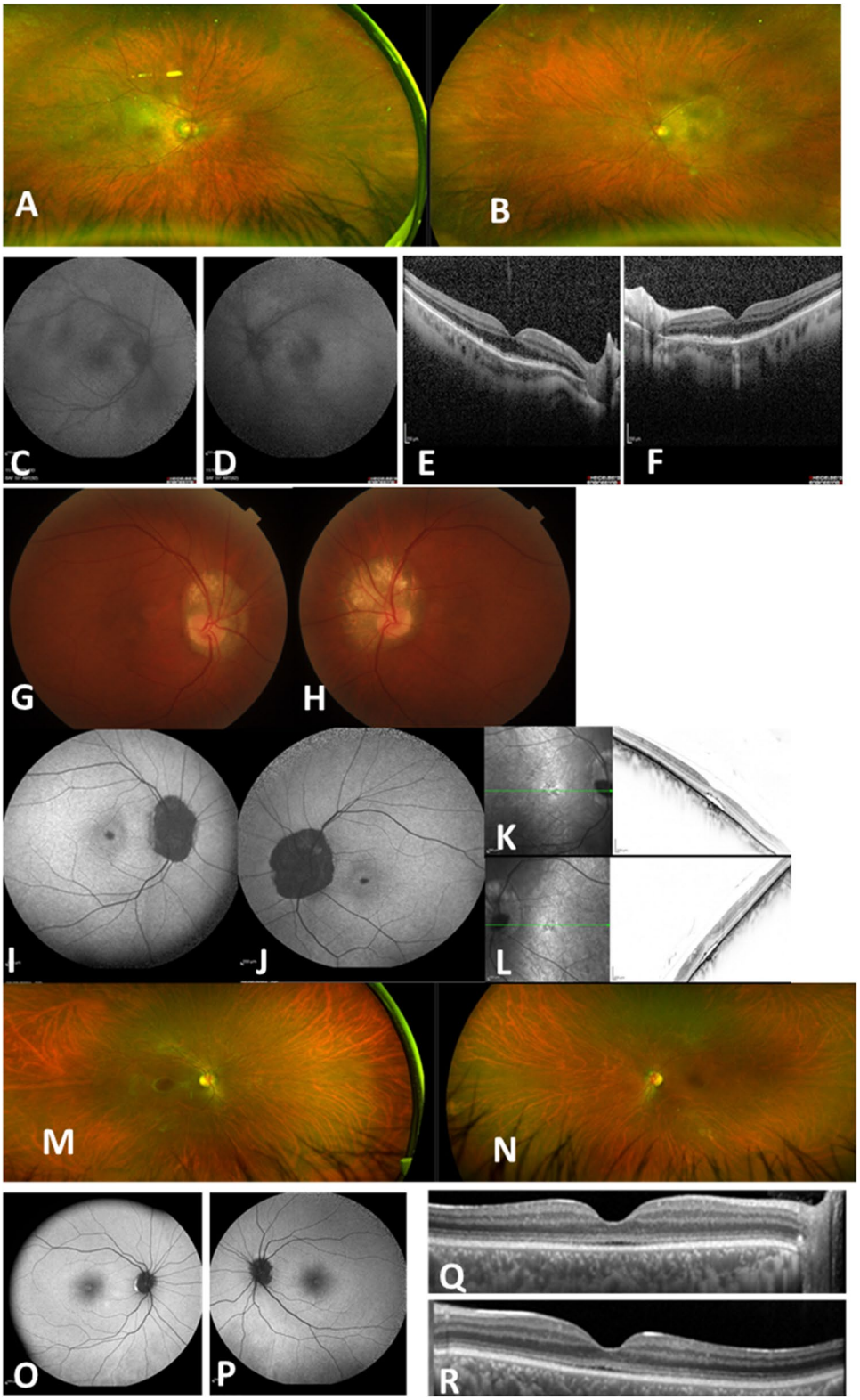


**Fig. 1** Funduscopic, autofluorescence, and SD-OCT characteristics of patient #1 carrying a mutation in *UNC119* (**A**–**F**), patient #2 carrying *CNGA3* compound heterozygous mutations (**G**–**L**), and patient #3 carrying *POC1B* compound heterozygous mutations (**M**–**R**). Detailed description is provided in Table 1

c.70 T > C (p.Trp24Arg) mutation in the *BEST1* gene was recognized through NGS analysis in this patient. Parental DNA analysis evidenced that both healthy parents were heterozygous carriers of the c.70 T > C variant in *BEST1*.

## Patient #5

This 39-year-old male complained of central blurry vision and nyctalopia since he was 18. He had a 37-year-old sister

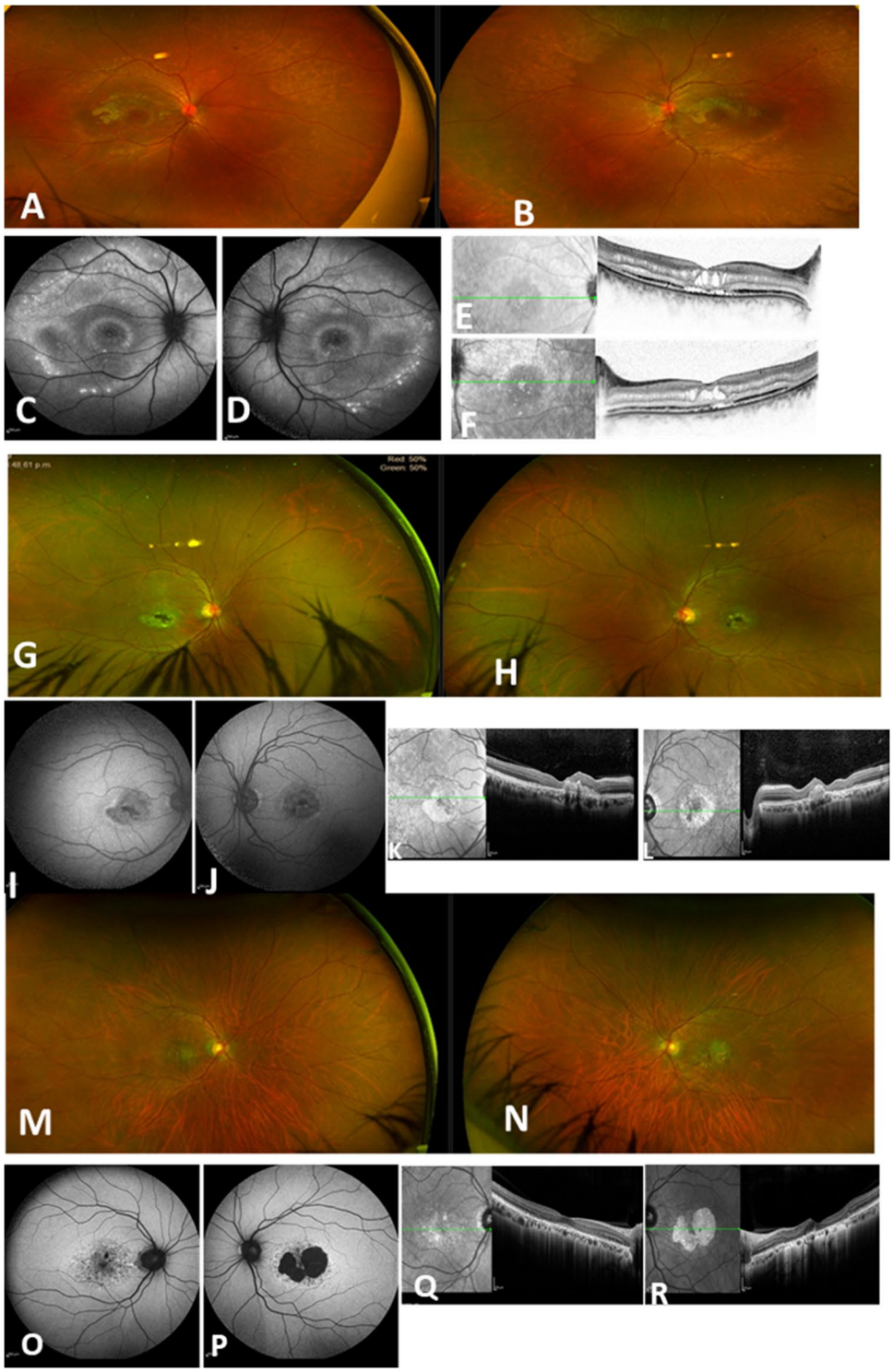


**Fig. 2** Funduscopic, autofluorescence, and SD-OCT characteristics of patient #4 carrying a homozygous *BEST1* mutation (**A**–**F**), patient #5 with a homozygous *CYP2U1* mutation (**G**–**L**), and patient #8 carrying a *PRPH2* mutation (**M**–**R**). Detailed description is provided in Table 1

with similar symptomatology and ophthalmological findings. His parents were third-degree cousins (Supplementary Fig. 4). BCVA was 0.6 logMAR (0.25 Snellen decimal) in the right eye and 0.7 logMAR (0.2 Snellen decimal) in the left eye. Fundus examination showed a macular scar and hyperpigmentation surrounded by a hypopigmented ring in both eyes (Fig. 2G–L). No systemic anomalies or neurological symptoms were recorded on examination. Exome sequencing allowed the recognition of a previously reported homozygous c.1168C > T (p.Arg390Ter) mutation in the *CYP2U1* gene.

### Patient #6

This 37-year-old male complained of progressive bilateral loss of visual acuity for at least 5 years. No family history of visual defects was recorded. His BCVA was 0.1 logMAR (0.8 Snellen decimal) in both eyes. During funduscopy, macular hypopigmentation and hyperpigmented spots with parafoveal confluence were observed bilaterally. Genetic analysis in this subject identified a previously reported c.749G > A (p.Cys250Tyr) heterozygous mutation in the *PRPH2* gene.

### Patient #7

This 42-year-old male has complained of blurry vision since childhood. Since the age of 22, he has had progressive visual loss with no improvement with the use of eyeglasses. Photophobia, inability to distinguish certain shades of color, and decreased central vision were also referred. BCVA was 1.82 logMAR (0.63 Snellen decimal) in the right eye and 1.82 logMAR (0.63 Snellen decimal) in the left eye. Fundus examination showed macular retinochoroidal atrophy patches surrounded by discrete yellowish, round, and pisciform flecks and peripapillary atrophy in both eyes. NGS analysis disclosed compound heterozygosity for two previously described mutations in the *PROM1* gene: c.1354dupT (p.Tyr452fs) and c.1984-1G > T. Familial Sanger sequencing analysis confirmed the *trans* configuration of the variants.

### Patient #8

A 47-year-old female complaining of metamorphopsia since several months ago with no other ocular symptoms was evaluated. She had received a previous diagnosis of neovascular membrane secondary to age-related macular degeneration and was treated with a dose of intravitreal aflibercept in the left eye. No family history related to a similar eye condition was recorded, although her late father had worn high-grade eyeglasses. At present, her BCVA was 0.9 logMAR (0.125 Snellen decimal) in the right eye and 0.17 logMAR (0.63 Snellen decimal) in the left eye. Fundus examination showed a macular scar on the left eye, and atrophy and drusen in both eyes (Fig. 2M–R). In this subject, exome sequencing evidenced a previously reported c.424C > T (p.Arg142Trp) heterozygous mutation in the *PRPH2* gene.

## Discussion

As a retinal dystrophy subtype, maculopathies are of special interest as they are consistently associated with early and clinically relevant central vision deficiency. Stargardt disease and Best macular dystrophy, the two most commonly occurring monogenic maculopathies, exhibit high allelic heterogeneity, as approximately 1700 different disease-associated mutations in *ABCA4* and approximately 380 in *BEST1* genes have been identified, according to the Human Gene Mutation (HGMD, http://www.hgmd.cf.ac.uk/) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases, accessed on August 2021. However, MD can be caused by defects in rare genes that are not routinely analyzed or not included in NGS gene panels. For example, *POC1B* and *CYP2U1* genes are not included in a panel of 2742 genes related to monogenic diseases that we had used previously to characterize causal mutations in a large cohort of retinal dystrophy patients [28].

In this work, we presented the phenotypic and genetic findings in a group of eight unrelated Mexican individuals suffering from different forms of retinal disease arising from mutations in known maculopathy genes. Exome sequencing allowed the recognition of the molecular cause of the disease in all cases, demonstrating the utility of this approach for genetic diagnosis in heterogeneous disorders. While WES has proven to be effective for molecular diagnosis of genetically heterogeneous diseases, its limitations include the inability to identify noncoding regulatory or deep intronic regions as well as genomic structural variation.

Of the 11 pathogenic alleles identified, three were previously unreported mutations, thus expanding the current knowledge of the molecular spectrum causing inherited maculopathies.

In patient #1, a causal c.601G > T (p.Glu201Ter) heterozygous variant at the *UNC119* gene was identified. *UNC119* encodes a protein localized to the photoreceptor synapses in the outer plexiform layer of the retina, where it has been suggested to play a role in the mechanism of photoreceptor neurotransmitter release through the synaptic vesicle cycle [29]. Previously, Kobayashi et al. recognized a UNC119 heterozygous p.Lys57Ter nonsense mutation in an adult Japanese subject with cone-rod dystrophy. This patient complained of poor night vision, defective color vision, and photophobia from the age of 40 years and exhibited macular atrophy, pericentral ring scotomas, and abnormal ERG consistent with cone-rod dystrophy [30]. Interestingly, transgenic mice carrying an identical p.Lys57Ter mutation exhibited a progressive decrease in the ERG b-wave associated with retinal degeneration with prominent degeneration of the synapses [30]. Huang et al. reported a child suffering from photophobia and exhibiting atrophy and pigmentation deposits of central macula in whom a heterozygous p.Asp87Asn UNC119 missense mutation was demonstrated [31]. More recently a c.7delG (p.Val3$^{*}$) was reported in a family with dominant retinitis pigmentosa [32]. Thus, the likely pathogenic p.Glu201Ter UNC119 variant described here is the fourth retinopathy-related *UNC119* mutation, supporting the involvement of this gene in hereditary macular disease.

In patient #2, compound heterozygosity for the c.1541A > T (p.Asp514Val) and c.1981C > A (p.Arg661Ser)

variants in the *CNGA3* was demonstrated. CNGA3 encodes the alpha subunit of the cone photoreceptor cyclic nucleotide-gated (CNG) channel, which is a crucial component of the phototransduction cascade in cone outer segments [33]. Mutations in *CNGA3* account for approximately 25% of either complete or incomplete cases of achromatopsia, an uncommon, autosomal recessive inherited retinal condition manifesting shortly after birth or early infancy and characterized by reduced visual acuity, nystagmus, photophobia, and very deficient or absent color vision [34, 35]. Achromatopsia can result from mutations in at least six genes (i.e., *CNGA3*, *CNGB3*, *GNAT2*, *PDE6C*, *PDE6H*, and *ATF6*), which are crucial for phototransduction in cones [24, 36].

In patient #3, compound heterozygosity for c.101-3 T > G (splicing) and c.144delG (p.Lys48AsnfsTer16) mutations in the *POC1B* gene was demonstrated. *POC1B* encodes a protein required for centriole integrity, basal body stability, and ciliogenesis of the photoreceptors [37, 38]. To date, 24 sporadic or familial macular dystrophy cases carrying biallelic *POC1B* mutations have been reported (see the supplementary file for references). In virtually all cases, a cone dystrophy phenotype is observed with decreased central vision, early-onset photophobia and dyschromatopsia, unrecordable ERG photopic responses, and disruption of the ellipsoid zone on OCT. Most individuals carrying biallelic *POC1B* mutations have normal funduscopic appearance, even at adult ages. POC1B mutations p.Thr119Ile and pArg452Gln are frequent in Japanese patients [39] while mutation p.Arg106Pro has been identified in families from the Middle East [40, 41]. Previous in vitro studies indicated that the c.101-3 T > G variant in POC1B produces two aberrant transcripts that are out-of-frame and would cause a premature truncation of the protein [22].

A homozygous c.70 T > C (p.Trp24Arg) mutation in *BEST1* was demonstrated in patient #4. Genetic defects in *BEST1* cause a variety of retinal phenotypes, particularly autosomal dominant Best disease, as well as autosomal dominant vitreoretinochoroidopathy and microcornea, rod-cone dystrophy, cataract, and posterior staphyloma syndrome (OMIM #193220), retinitis pigmentosa (OMIM #613914), and autosomal recessive bestrophinopathy (ARB) (OMIM #611809) (reviewed in [42]). ARB patients exhibit multifocal yellow subretinal deposits, subretinal fluid, macular edema, hyperopia with short axial-length, and angle closure [43, 44]. Thus, the observed phenotype in our patient is fully compatible with ARB.

Patient #5 harbored a homozygous c.1168C > T (p.Arg390Ter) mutation in *CYP2U1*, a gene encoding for protein P450, an enzyme involved in the hydroxylation of long chain fatty acids and phospholipid degradation [45]. *CYP2U1* mutations underlie spastic paraplegia 56 (SPG56), an autosomal recessive neurodegeneration characterized by early-onset progressive lower-limb spasticity and weakness [46]. It is noteworthy that a pigmentary degenerative maculopathy has been observed as part of the SPG56 phenotype [23]. No neurological symptoms were recognized in our patient carrying the p.Arg390Ter variant nor in his affected sister, and this observation is in agreement with recent data indicating that *CYP2U1* mutations can cause maculopathy without SPG56 neurological signs [Vaclavik V, et al. IOVS 2019;60:ARVO E-Abstract 2930]. The CYP2U1 p.Arg390Ter mutation described here is identical to that demonstrated in a familial SPG56 Italian pedigree [23]. Thus, available evidence supports that *CYP2U1* can be also considered as an isolated maculopathy gene.

Heterozygous mutations in the *PRPH2* gene were demonstrated in patients #6 (c.749G > A; p.Cys250Tyr) and #8 (c.424C > T; p.Arg142Trp). *PRPH2* encodes a photoreceptor-specific tetraspanin glycoprotein, PRPH2 (also known as peripherin/RDS), a main structural component of the photoreceptor outer segment [47]. Disease-causing variants in *PRPH2* are associated with a diversity of phenotypes including cone-rod dystrophy, Stargardt disease, pattern dystrophy, and retinitis pigmentosa. Patients with *PRPH2*-related MD usually exhibit various hypo- or hyper-pigmented regions that appear as focal or multi-focal yellow, orange, or gray spots on the fundus [48]. A recent survey indicated that there are up to 252 different mutations in this gene associated with diverse retinal phenotypes [49].

Patient #7 was found to be compound heterozygous for mutations c.1354dupT (p.Tyr452Leufs*13) and c.1984-1G > T and in *PROM1*. *PROM1* encodes prominin-1, a member of a family of 5-transmembrane domain proteins with a critical role in disk morphogenesis and autophagy of the retinal pigment epithelium [50]. According to the HGMD public database and ClinVar (accessed in August 2021), there are at least 70 distinct disease-causing variants in *PROM1* underlying a spectrum of retinal conditions, particularly autosomal dominant-cone-dominant disease including macular dystrophy, cone-rod dystrophy, and Stargardt-like macular dystrophy. Although variable, the *PROM1*-associated phenotype can include severe macular involvement with peripheral bone-spicule degeneration [51], atrophic macular changes with foveal sparing, and generalized cone dysfunction on ERG [52]. In the patient reported here, the identification of biallelic *PROM1* mutations indicated a diagnosis of recessive cone-rod dystrophy type 12 (OMIM #612657).

In summary, we presented the clinical-genetic findings in a group of MD patients in which exome sequencing efficiently identified causal mutations in known maculopathy genes. Our results expand the current knowledge of the allelic spectrum linked to monogenic maculopathies.

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**Author contribution** The authors confirm their contributions to the paper as follows: study conception and design: JCZ; data collection: R A-G, RM, A L-B, LM, A M-A, OF CH-C; analysis and interpretation of results: JCZ, R A-G, LM, A M-A, OF CH-C; draft manuscript preparation: JCZ, RM, OF CH-C; supervision and review of the manuscript: JCZ, OF CH-C. All authors reviewed the results and approved the final version of the manuscript.

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## Declarations

**Ethics approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics and Research Committees of the Institute of Ophthalmology "Conde de Valenciana" and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

**Consent to participate** Informed consent was obtained from all individual participants included in the study.

**Conflict of interest** The authors declare no competing interests.

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