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Novel *RCBTB1* variants causing later-onset non-syndromic retinal dystrophy with macular chorioretinal atrophy

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Abstract

Background: Variants in *RCBTB1* were recently described to cause a retinal dystrophy with only eight families described to date and a predominant phenotype of macular atrophy and peripheral reticular degeneration. Here, we further evaluate the genotypic and phenotypic characteristics of biallelic *RCBTB1*-associated retinal dystrophy in a North American clinic population.

Methods: A retrospective analysis of genetic and clinical features was performed in individuals with biallelic variants in *RCBTB1*.

Results: Three unrelated individuals of French-Canadian descent with rare biallelic *RCBTB1* variants were identified. All individuals shared a novel p.(Ser342Leu) missense variant; one patient was homozygous whereas the other two each possessed a second unique novel variant p.(Gln120*) and p.(Pro224Leu). All three had macular-predominant disease with symptom onset in the fifth decade of life.

Conclusion: This report adds to the genetic diversity of *RCBTB1*-associated disease. These cases confirm the later-onset, relative to many other retinal dystrophies, and macular focus of disease described in most cases to-date. They are thus a reminder of considering hereditary disease in the differential for later-onset macular atrophy.

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Keywords

macular atrophy; *RCBTB1* ; retinal dystrophy

INTRODUCTION

Inherited retinal dystrophies (IRDs) are a genetically and phenotypically diverse group of diseases that affect more than 2 million individuals worldwide and result in significant visual disability and blindness(1, 2). Mutations in nearly 300 genes are known to cause these conditions (https://sph.uth.edu/retnet/) and while mutations in certain genes account for a significant fraction of disease, the remaining genetic forms are rare. Although many disease-causing genes have roles in retina-specific pathways, an increasing number of genes are being identified with more ubiquitous roles in tissue homeostasis and stress response (https://sph.uth.edu/retnet/).

RCBTB1 is a protein that comprises a regulator of chromosome condensation 1 (RCC1)-like domain (RLD) and two broad complex, tramtrack, and bric-a-brac (BTB) domains(3). It has been proposed to have a function in cell cycle regulation(3), and some evidence points to its potential role as a tumor suppressor gene(4). Expression array data on total RNA samples has shown ubiquitous expression in all human adult tissues(5). Targeted analysis of expression has revealed relatively high expression of *RCBTB1* mRNA in human neural retina compared to retinal pigment epithelium (RPE)(5). In the retina specifically, it has been hypothesized that RCBTB1 may act as a substrate adaptor in the ubiquitinylation pathway(6) and possibly modify the localization of oxidative stress-response transcription factors(5, 7).

At the time of writing, only eight IRD families have been reported with retinal disease due to mutations in *RCBTB1* (OMIM 607867)(5, 8–10). The available phenotypic data from reports of retinal disease suggests that although mutations in *RCBTB1* can cause typical retinitis pigmentosa (RP), the more common presentation may be a later-onset retinal dystrophy characterized by prominent macular atrophy(5, 8, 9). Here, we describe three unrelated patients of French-Canadian descent with recessive disease and carrying novel pathogenic *RCBTB1* variants. This series increases the number of reported patients with non-syndromic disease and describes a novel variant shared between the two compound heterozygous patients. All three of these patients have later-onset disease with functional consequences due to macular involvement.

METHODS

This retrospective study was conducted at Massachusetts Eye and Ear (MEE) under a protocol approved by the local institutional review board. The study met the tenets of the Declaration of Helsinki. Individuals with two rare, likely biallelic variants in *RCBTB1* were identified from the patient population of a clinic specializing in inherited retinal dystrophies.

A. Genetic Analysis

Blood samples were obtained from probands and available family members, and DNA was isolated from peripheral blood lymphocytes by standard procedures. Genetic testing was

performed through the MEE Ocular Genomics Institute for two subjects (OGI1042_002062 and OGI3572_005183) and by a commercial diagnostic genetic testing lab for the third (OGI3572_005182). For testing performed at MEE, the Genetic Eye Disease panel and previously described analysis methods were used(11). Guidelines of the American College of Medical Genetics(12) were used for the interpretation of sequence variants. Variants were verified to have an allele frequency of less than 1% in the Genome Aggregation Database (gnomAD)(13). The Combined Annotation Dependent Deletion (CADD) tool including PHRED-scaling was used to score the deleterious of the missense variants with scores of greater than 20 suggesting that a variant is more likely to have a deleterious effect(14). Predictions from in silico modeling including SIFT(15), PolyPhen-2(16), and MutationTaster(17) were also used to help assess variant pathogenicity. Finally, structural modeling was used to assess the impact of missense mutations on polar bond organization and surrounding structure. Tridimensional structure of the RCBTB1 protein was generated with a protein modelling software (PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC) using AlphaFold Database-predicted(18) (https://alphafold.ebi.ac.uk) structure of human RCBTB1 as an input (AlphaFold ID: AF-Q8NDN9-F1).

When available, DNA samples were obtained from family members for segeregation analysis.

B. Clinical evaluation

All clinical assessments were performed in the Massachusetts Eye and Ear (MEE) Inherited Retinal Disorders Service. Visual acuity was assessed using Snellen charts. Kinetic perimetry was evaluated with Goldmann perimetry. Full-field ERGs were conducted with Burian Allen electrodes and a custom ERG system(19). Fundus exam was complemented by fundus photography (Optos 200Tx and California devices, Optos PLC, Dunfermline, Scotland, United Kingdom), widefield fundus autofluorescence imaging (Optos), and spectral-domain optical coherence tomography (SD-OCT: Spectralis, Heidelberg Engineering, Heidelberg, Germany). The region tool within the Heidelberg Spectralis software was used to outline central macular areas of disrupted near infrared reflectance on high resolution images for Patient 1 (Heidelberg Engineering Region Finder Module 2.6.1.0).

RESULTS

A. Genetic analysis

Three unrelated patients (2 males, 1 female) with biallelic rare variants in *RCBTB1* were identified (Table 1). One of the variants led to a premature stop codon in exon 5 (of 13 exons total): c.358C>T, p.(Gln120*), likely leading to degradation of the transcript and no protein product. The remaining two *RCBTB1* variants found were missense changes that fell within exons encoding the RCC1-like domain (Figure 1, Tables 1 and 2). The CADD-PHRED scores for the missense variants were greater than 20 thus suggesting a deleterious effect (p.(Pro224Leu) C-score=31; p.(Ser342Leu) C-score=22.9). These scores for the missense variants, as well as combined information from in silico predictions and ACMG classification of each of the three variants (Table 2), supported pathogenicity.

Structural modeling of the two missense variants found that they did not affect polar bond organization or significantly modify surrounding protein structure, but the analysis did not permit any conclusions regarding whether these variants impaired binding efficiency with other proteins (data not shown).

None of the variants have been reported before in association with retinal disease; the missense variants are reported in gnomAD but not in the homozygous state, and the variant with the premature stop is absent in gnomAD. One missense variant (c.1025C>T, p.(Ser342Leu)) was shared by all three individuals, and it was homozygous in one patient (Patient 3, OGI3572_005183; no testing of family members performed) and *in trans* with the c.358C>T, p.(Gln120*) variant in another (Patient 1; OGI1042_002062; confirmed by biparental testing). Family members were not available for testing in Patient 2 (OGI3572_005182), but the gnomAD Variant Co-Occurrence calculator predicts these two variants occur on different haplotypes supporting that these variants are likely in trans(20). All three patients reported French-Canadian ancestry. Two of Patient 2's four siblings had subjective difficulty with night vision and one carried a possible diagnosis of a retinal degeneration (Supplemental Figure 1). There was no family history of retinal disease or degeneration in the other two patients. Variants in other IRD genes (https://sph.uth.edu/retnet/) were found for two patients (Supplemental Table 1), but none of the variants detected were able to explain the phenotype in the reported cases.

B. Clinical data

Clinical assessments, with data summarized in Table 1, were performed at a single visit for two patients and spanned three years for one individual (Patient 1). Patients were between 46 and 62 years of age at initial examination. They described symptoms of decreased central vision, increased glare, and nyctalopia with most of these beginning when they were in their early to mid-40's. Patient 3 was told that he had retinal abnormalities during a routine exam at age 25 suggesting that anatomic changes predated any functional impact. At the time of initial exam, visual acuity ranged from 20/20 to "count fingers" with an accompanying range of macular disruption apparent on fundus exam and with retinal imaging. The oldest patient (Patient 3) had the most impaired visual acuity at the initial exam.

Fundus exam in all individuals showed macular atrophy of varying severity and characterized by nummular areas of atrophy that could be either fovea-sparing or fovea-threatening even in earlier stages of macular disease and later becoming confluent (Figure 2, A–C). Abnormalities of the peripheral retina varied in extent and included subtle pigment mottling (Patient 1), fine pigment deposits (Patient 2, 3), and chorioretinal atrophy (Patient 2). At least one patient had a reticular pattern of pigment deposition in the peripheral retina. Fundus autofluorescence (Figure 2, D–F) emphasized the distribution of clinically-apparent atrophy but was also more diffusely peripherally disrupted in two patients (Patients 2, 3). In addition to delineating areas of clear atrophy, OCT showed reduced definition of the RPE and ellipsoid zone even in seemingly intact areas of retina (Figure 2, G–H). Numerous small cystoid spaces were present in the inner and outer retina overlying areas of atrophy in Patient 2. Retinal imaging over an approximately three-year interval in Patient 1 illustrated significant progression of macular involvement that corresponded to visual acuity decreasing

from 20/20 in the right eye and 20/25 in the left eye to 20/30 and 20/600 respectively (Figure 3). The area of abnormal reflectance on near infrared imaging corresponding to the atrophy expanded by 1.1 mm^2/year in the right eye and 1.2 mm^2/year in the left eye (Starting values: 2.18 mm^2 OD, 3.24 mm^2 OS; final values 3.24 mm^2 OD, 6.96 mm^2 OS).

Goldmann perimetry demonstrated relative central scotomas but full peripheral fields. Full-field ERG demonstrated normal to mildly abnormal rod- and cone-isolated responses, but even when abnormal, responses remained robust (Table 1).

Patient 2 had additional medical diagnoses of tremor, anxiety, and nephrolithiasis; Patients 1 and 3 were in good health.

DISCUSSION

In this cohort of patients with *RCBTB1*-associated retinal dystrophy, we thoroughly characterize the clinical phenotype and identify three novel disease-causing variants including a suspected founder mutation in the French-Canadian population. The functional (perimetry, ERG) and anatomic (widefield color, autofluorescence, OCT) descriptions of this cohort complement prior reports but also provide new information on the clinical spectrum of disease. Our description of prominent macular atrophy and peripheral pigmentary abnormalities corresponds to the findings described in five of six families in the original report of *RCBTB1*-associated disease(5) as well as in available data from two additional individuals(9, 21). Features of more typical retinitis pigmentosa were present in one family(5) but appear to be a phenotypic outlier in *RCBTB1*-associated disease. Full-field ERG recordings in the current cohort demonstrated the potential for varying degrees of rod and cone dysfunction consistent with the range of changes previously reported (21). Even in the patient with the most appreciably impacted ERGs (Patient 1), however, the response amplitudes were better preserved than those seen in typical RP. Indeed, the impact on visual acuity and central perimetry was consistent with the macular focus of degeneration. The progression over a three-year interval from 20/20 to counting fingers acuity in Patient 1 demonstrates the rapidity with which fovea-threatening disease can impact functional vision. The rate of atrophy expansion was similar to what has been described previously(21).

The typical *RCBTB1*-associated cases in our cohort and others are characterized by central vision symptoms appearing and progressing in a patient's 40s or 50s, with subsequent onset of nyctalopia. In this respect, the disease is similar to other later-onset disorders that can have significant impact on central vision through macular involvement, such as a *C1QTNF5*-associated late-onset retinal dystrophy (LORD)(22, 23) or autosomal dominant *RPE65*-associated retinal degeneration(24). In conjunction with reticular peripheral retinal pigmentation, the potential overlap with nonexudative age-related macular degeneration is also apparent; this diagnosis, and inherited retinal dystrophies more generally, should be considered in younger patients with macular atrophy but no drusen. However, as previously described, there are several examples of genes (e.g., *PRPH2*(25, 26), *ABCA4*(27)) in which mutations can cause dystrophies that vary from macular-dominant disease to features consistent with typical RP. Our cases highlight the importance of including evaluation of *RCBTB1* in genetic analysis of patients with apparently non-syndromic later-onset retinal

dystrophy. Finally, unlike a previous report(5) which detailed 7 cases with associated systemic findings, our small cohort also did not have features suggestive of syndromic disease; they thus add to 5 of the 12 previously reported cases of *RCBTB1*-associated retinal dystrophy with non-syndromic disease(5, 8, 9).

The three novel *RCBTB1* variants found in this cohort expand the list of disease-associated variants. All three patients, from unrelated pedigrees, shared the c.1025C>T, p.(Ser342Leu) variant. Even though all three patients self-reported French-Canadian ethnicity, this variant is predominantly present in Finnish individuals in gnomAD and therefore, at present, it is unclear if the c.1025C>T p.(Ser342Leu) change is a founder allele or a mutational hot-spot. The p.(Ser342Leu) change affects the sixth RCC repeat of the RCC1-like domain, which harbors three additional missense variants associated with disease (Figure 1, Table 2), and the surrounding amino acids are highly conserved throughout evolution(5). Patient 3 who was homozygous for the c.1025C>T p.(Ser342Leu) mutation had the earliest onset and most anatomically-advanced phenotype of the three cases, which may also be related to the fact that he was more than a decade older than the other cases. Of the remaining two patients, one carried a null allele in exon 5 (c.358C>T, p.(Gln120*)) and the other carried a variant affecting the fourth RCC repeat (c.671C>T, p.(Pro224Leu)). Given the limited number of cases in the literature, additional cases will be needed to determine whether there is any genotype-phenotype correlation in age-of-onset or disease severity.

Initial insights regarding the molecular function of *RCBTB1* came from oncology with a focus on cell cycle regulation. The identification of *RCBTB1* in 2001 was facilitated by the commonly encountered deletion of chromosome 13q14, the region within which it falls, in B-cell chronic lymphocytic leukemia(3). The RCBTB1 protein was proposed to have a function in cell cycle regulation based upon the two protein domains encoded within its sequence (regulator of chromosome condensation-like (RCC1); broad complex, tramtrack, and bric-a-brac (BTB))(3). Indeed, the function of *RCBTB1* as a tumor suppressor gene has been supported by findings including decreased *RCBTB1* transcript levels in multiple cancer cell lines and the activation of DNA repair pathways following its expression(4). *RCBTB1* expression levels influence resistance to chemotherapy-induced apoptosis in cell lines with a clinical correlate of an association between deletion and metastatic progression in patients with sarcomas, particularly in individuals with prior chemotherapy(4, 28). Increasing *RCBTB1* levels by inhibiting the microRNA miR-26a-2 increases susceptibility to chemotherapy-induced apoptosis in a liposarcoma cell line and thus offers one potential method of modifying expression levels of this gene(28).

The precise retinal functions of *RCBTB1* remain under investigation. A more specific role for *RCBTB1* within the ubiquitination pathway is emerging that has implications for understanding the mechanisms of *RCBTB1*-associated retinal disease. Protein tagging with ubiquitin, through the action of ubiquitinylation enzymes (E1, E2, E3), targets the complex to the proteasome for degradation or other cellular processes. RCBTB1 interacts with this pathway in two ways: it is proposed to be a substrate adaptor for a cullin3 (CUL3) E3 ligase, and it also binds to the mouse homolog of UBE2E3, an E2 ubiquitin-conjugating enzyme, through an interaction facilitated by the BTB domain(6). *UBE2E3,* like *RCBTB1,* is expressed in the retina and is important for mediating the localization and activity of the

stress-response transcription factor *NRF2*(7). Loss of *NRF2* is known to play a role in retinal vulnerability to oxidative stress(29, 30). Findings by Coppieters et al suggest how *RCBTB1*-associated retinal disease might impact pathways important for regulating oxidative stress and suggest points for intervention. Individuals with *RCBTB1*-associated retinal dystrophy showed lower levels of *NRF2* expression than control individuals based on mRNA expression in peripheral blood mononuclear cells(5). As previously noted(5), however, the pathologic accumulation of other proteins destined for degradation in the proteasome cannot be excluded as a disease mechanisms in *RCBTB1*-associated retinopathy(31, 32). RPE cells derived from induced pluripotent stem cells (iPSCs) in individuals with *RCTB1*-associated retinal disease are also beginning to provide insights into aberrations at a retina-specific level(33).

If the mechanism of *RCBTB1*-associated disease is one of accumulation of oxidative damage, then multiple therapeutic strategies might be envisioned. Animal studies in which *NRF2* was overexpressed using an AAV-vector in mouse models of RP showed prolonged cone survival(34). Oral neuroprotective strategies based on preventing oxidative damage may also have therapeutic potential. For example, N-acetyl cysteine was previously shown to reduce oxidative damage and increased cone function and survival in animal models of RP(35), and a recent report of a Phase I clinical trial for oral N-acetylcysteine suggested that it was safe, well-tolerated and may improve the function of macular cones in patients with moderately-advanced RP(36). It remains to be seen whether these strategies based on reducing oxidative damage may have a differential, genotype-specific, effect based on a specific pathogenic mechanism for particular IRDs. Recent work also in RPE derived from iPSCs from a patient with *RCBTB1*-associated retinopathy also suggests the potential benefits of AAV-mediated *RCBTB1* gene augmentation(33).

CONCLUSION

Our report provides a comprehensive description of the phenotype of three cases of retinal dystrophy of varying severity associated with biallelic *RCBTB1* mutations and describes three new disease-causing variants. Three novel alleles are described including a potential founder mutation. This case series increases the number of reported families with nonsyndromic disease. The macular atrophy common to all three indivdiuals emphasizes the importance of considering retinal dystrophies in the differential of later-onset retinal disease. Further work is needed to elucidate the role of *RCBTB1* in the retina, both to understand retinal pathobiology of disease and to identify potential treatments which may intervene at the level of the gene or downstream effectors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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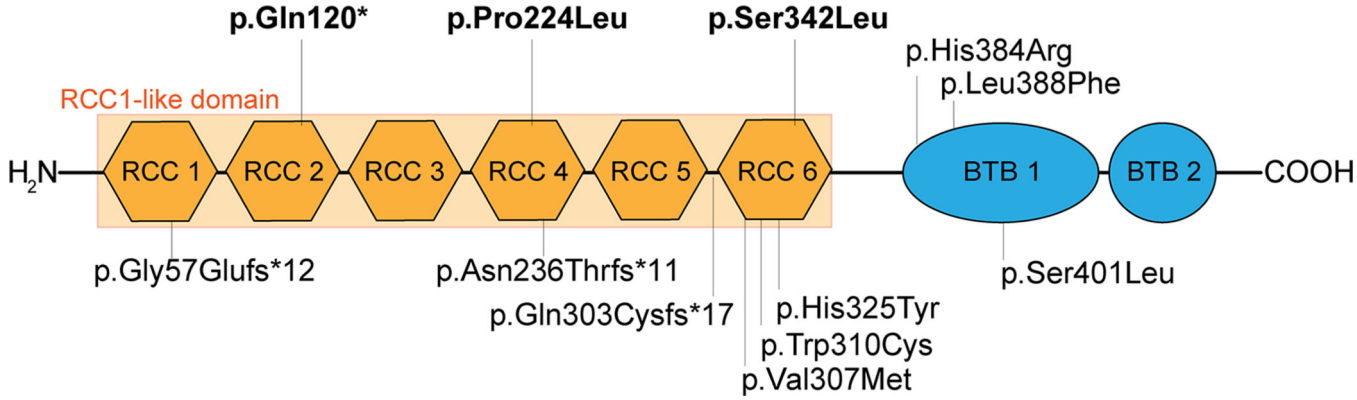

Figure 1: Location and features of disease-associated *RCBTB1* variants

The schematic figure shows the location of all the disease-associated variants in *RCBTB1* described in the literature and in this report. Novel variants are shown in bold font.

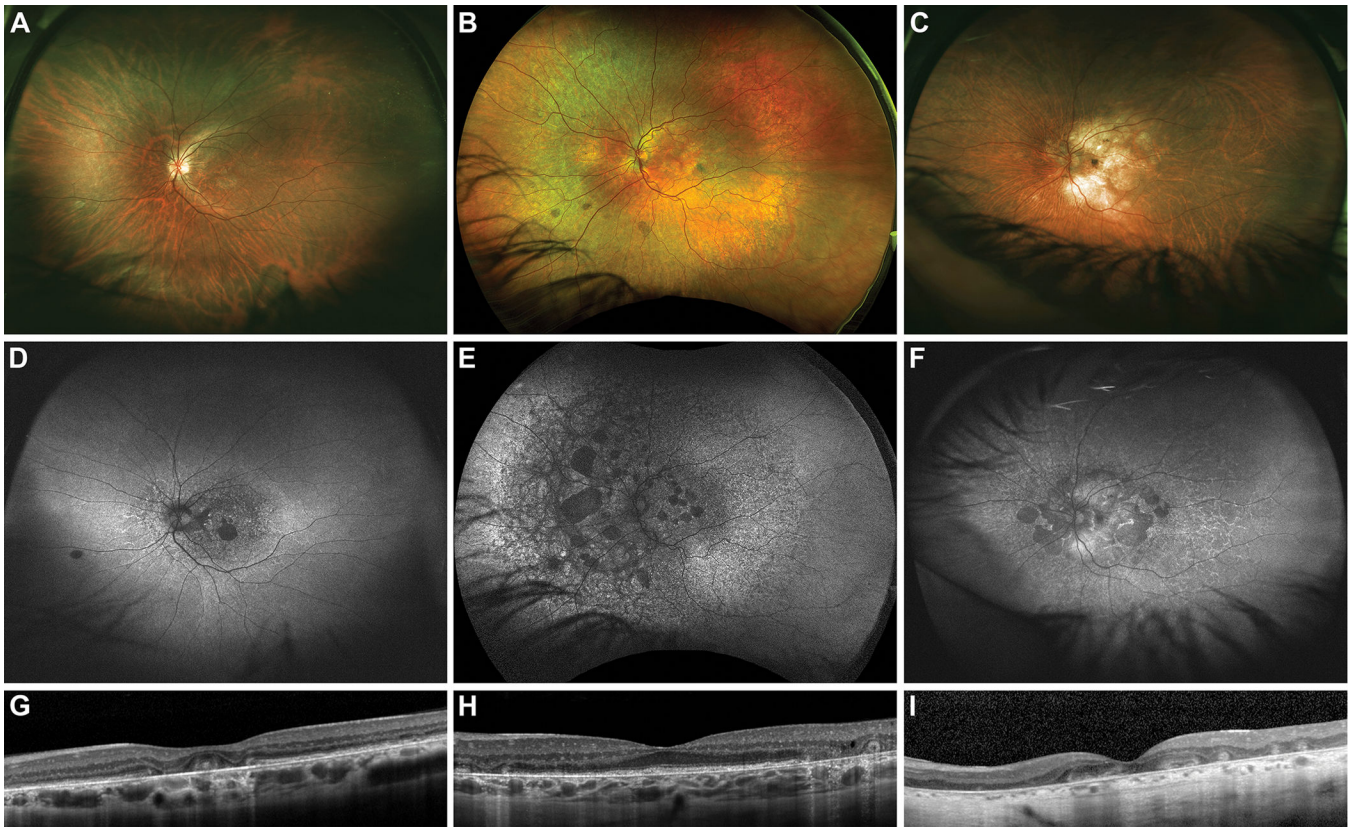

Figure 2: Fundus imaging of patients with *RCBTB1*-associated retinal dystrophy

Fundus photography (A-C), fundus autofluorescence (FAF; D-F), and SD-OCT (G-I) were obtained in all patients. In Patient 1, nummular patches of fovea-abutting atrophy were observed on exam and with fundus photography (A). Subtle pigment mottling was present in the midperipheral retina. FAF (D) highlighted the nummular nature of the atrophy and also showed generalized macular hypofluorescence without prominent peripheral disruption. SD-OCT (G) delineated areas of atrophy with a restricted island of foveal sparing in addition to showing more widespread RPE irregularities.

In Patient 2, fundus exam and photography (B) showed fovea-sparing macular atrophy. Fine pigment deposits, nummular areas of chorioretinal atrophy, and occasional larger pigment clumps were present in the peripheral retina. FAF (E) further illustrated the distribution of atrophy and pigment disruption. SD-OCT (H) showed generally intact foveal lamination. Areas of atrophy were also present with associated cystoid spaces and outer retinal tubulations (ORTs).

In Patient 3, fundus exam and photography (G) showed extensive macular atrophy with several prominent pigment clumps. Fine pigmentary deposits in the peripheral retina formed a reticular pattern in areas. FAF (H) showed coalescing nummular hypofluorescence in the macula with narrow strips of preserved signal. Reduced hypofluorescence was present throughout the midperiphery. Extensive chorioretinal atrophy and ORTs were present on SD-OCT (I) with limited areas of preserved outer retinal structure.

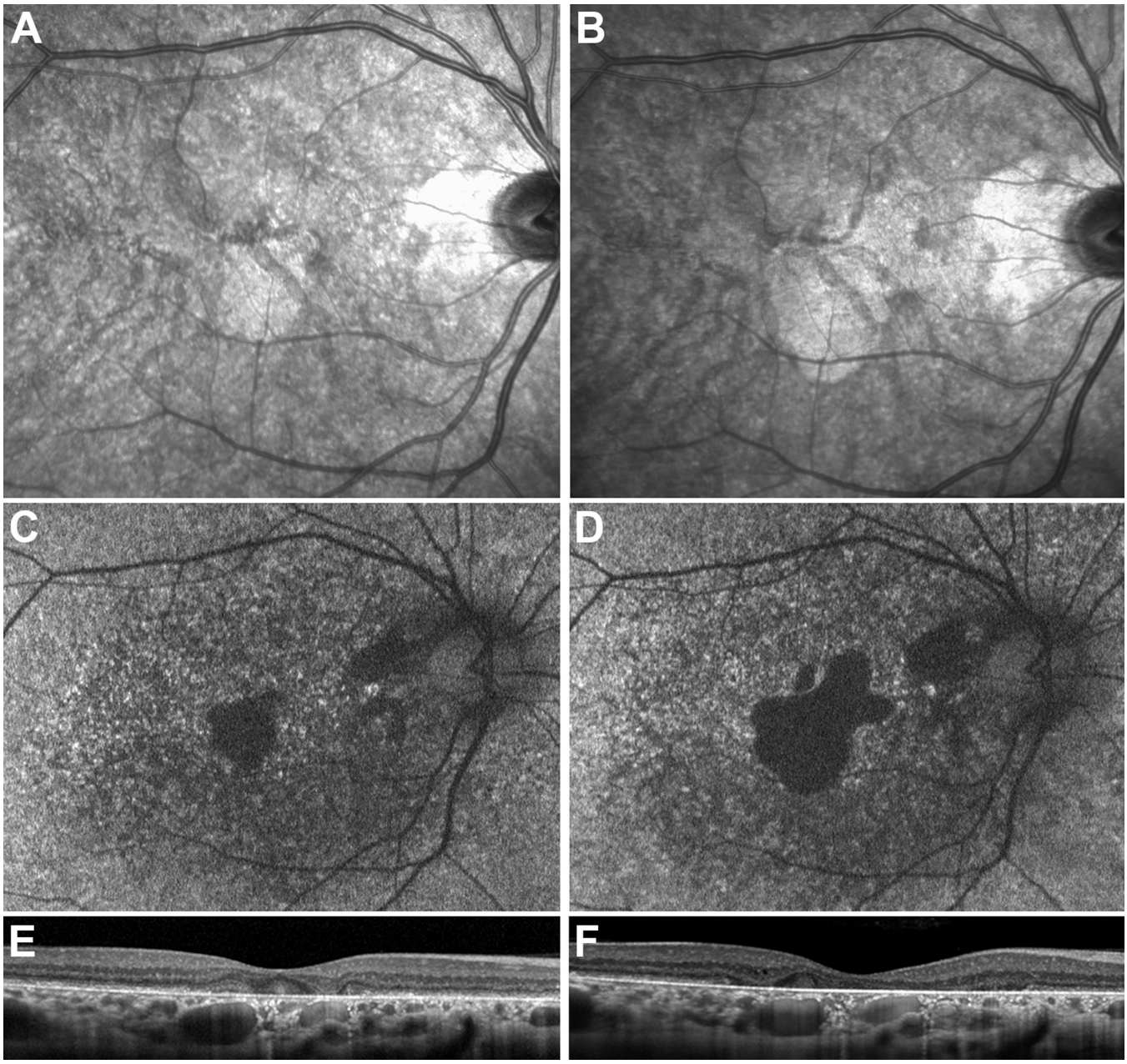

Figure 3: Evolution of macular findings in a single patient with *RCBTB1*-associated retinal dystrophy

Progressive macular disease was seen over a 3 year interval in Patient 1 as evaluated in near-infrared (A,B), fundus autofluorescence (C,D), and SD-OCT (E,F) imaging, presented for the right eye.

Table 1:

Genetic and clinical data

Patient ID / Gender	Variant 1[c]	Variant 2[c]	Symptoms (onset; y)	Age at exam (y)	Visual acuity	Goldmann perimetry	Electroretinogram
Patient 1[a] OGI1042_002062 Male	c.358C>T, p.(Gln120*)	c.1025C>T, p.(Ser342Leu)	Decreased central vision, glare (42)	46, 49	46y: OD 20/20 OS 20/25 49y: OD 20/30 OS 20/600	Relative central scotomas (≤8° to I4e), otherwise full	Rod-isolated: Reduced amplitude (~50–75% normal) Cone-isolated flicker: Reduced amplitude (30% of normal), normal timing
Patient 2[b] OGI3572_005182 Female	c.671C>T, p.(Pro224Leu)	c.1025C>T, p.(Ser342Leu)	Glare, nyctalopia (47)	49	OD 20/20 OS 20/20	Relative central scotomas to I2e only (≤6°); otherwise full	Rod-isolated: Reduced amplitude (~50% normal) Cone-isolated flicker: Normal amplitude and timing
Patient 3 OGI3572_005183 Male	c.1025C>T, p.(Ser342Leu)	c.1025C>T, p.(Ser342Leu)	Decreased central vision (45), nyctalopia (61)	62	OD CF 2 ft OS 20/40	Relative central scotomas (25–35° to I4e), full peripherally	Rod-isolated: Normal amplitude OD, borderline low amplitude OS Cone-isolated flicker: Borderline low amplitude with delayed timing OU

[a]Segregation analysis confirmed biparental inheritance

[b]Segregation analysis not available; gnomAD Variant Co-occurrence calculator used (see text)

[c]Variants are based on transcript NM_018191.4

Abbreviations: CF – count fingers; ft – feet; F – female; M – male; OD – right eye; OS – left eye; OU – both eyes; y - years

Table 2:

Disease-associated alleles reported to date

Variant[a]	gnomAD allele frequency	In silico predictions[b]	Protein domain[c]	ACMG classification[d]	Reference
c.170delG, p.(Gly57Glufs*12)	2.48e-5	Disease causing	RCC1	Pathogenic (PVS1, PM2, PP1-M)	Huang et al.(3); Yang et al.(4)
c.358C>T, p.(Gln120*)	Absent	Disease causing	RCC2	Likely Pathogenic (PVS1, PM2)	Current manuscript
c.671C>T, p.(Pro224Leu)	1.35e-5	Deleterious; Probably Damaging	RCC4	VUS (PM1, PM2_supporting, PM3_supporting, PP3)	Current manuscript
c.707delA, p.(Asn236Thrfs*11)	1.2e-4	Disease causing	RCC4	Pathogenic (PVS1, PM2, PP1-M)	Huang et al.(3)
c.905_906insTT, p.(Gln303Cysfs*17)	Absent	Disease causing	RCC5/6	Likely Pathogenic (PVS1, PM2)	Yang et al.(4)
c.919G>A, p.(Val307Met)	1.2e-5	Deleterious; Probably Damaging	RCC6	Likely Pathogenic (PM1, PM2, PP1-M, PP3)	Coppieters et al.(6)
c.930G>T, p.(Trp310Cys)	7.96e-6	Deleterious; Probably Damaging	RCC6	Likely Pathogenic (PM1, PM2, PP1-M, PP3)	Coppieters et al.(6)
c.973C>T, p.(His325Tyr)	1.42e-4	Tolerated; Possibly Damaging	RCC6	VUS (PM1, PM2, PP1)	Coppieters et al.(6)
c.1025C>T, p.(Ser342Leu)	1.1e-4	Tolerated; Benign	RCC6	Likely Pathogenic (PM1, PM2, PP1-M)	Current manuscript
c.1151A>G, p.(His384Arg)	9.22e-5	Deleterious; Probably Damaging	BTB1	Likely Pathogenic (PM1, PM2, PP1, PP3)	Coppieters et al.(6)
c.1164G>T, p.(Leu388Phe)	Absent	Deleterious; Benign	BTB1	Likely Pathogenic (PM1, PM2, PP1, PP3)	Coppieters et al.(6)
c.1202C>T, p.(Ser401Leu)	3.2e-5	Tolerated; Benign	BTB1	VUS (PM1, PM2, PP1)	Coppieters et al.(6)

[a] Variants are based on transcript NM_018191.4

[b] SIFT and PolyPhen-2 predictions are shown for missense variants; MutationTaster predictions are shown for variants that result in premature protein truncation.

[c] RCC: six repeats of RCC1(regulator of chromatin condensation 1)-like domain, BTB:

[d] American College of Medical Genetics (ACMG) variant classification (PVS1:null variant; PM1:variant in a functional domain; PM2: extremely rare; PM3: detected in *trans* with a pathogenic variant; PP1:variant segregates with disease in multiple affected individuals; PP1-M: same as PP1 with multiple families; PP3: multiple lines of computational evidence support a deleterious effect)

Abbreviations: VUS – variant of uncertain significance