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Whole Exome Sequencing Identifies *CRB1* Defect in an Unusual Maculopathy Phenotype

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Abstract

Objective—To report a new phenotype caused by mutations in the *CRB1* gene in a family with 2 affected siblings.

Design—Molecular genetics and observational case studies.

Participants—Two affected siblings and 3 unaffected family members.

Methods—Each subject received a complete ophthalmic examination together with color fundus photography, fundus autofluorescence (FAF), and spectral domain optical coherence tomography (SD-OCT). Microperimetry 1 (MP-1) mapping and electroretinogram (ERG) analysis were performed on the proband. Screening for disease-causing mutations was performed by whole exome sequencing in 3 family members followed by segregation analyses in the entire family.

Main Outcome Measures—Appearance of the macula as examined by clinical examination, fundus photography, FAF imaging, SD-OCT, and visual function by MP-1 and ERG.

Results—The proband and her affected brother exhibited unusual, previously unreported, findings of a macular dystrophy with relative sparing of the retinal periphery beyond the vascular arcades. The FAF imaging showed severely affected areas of hypoautofluorescence that extended nasally beyond the optic disc in both eyes. A central macular patch of retinal pigment epithelium (RPE) sparing was evident in both eyes on FAF, whereas photoreceptor sparing was documented in the right eye only using SD-OCT. The affected brother presented with irregular patterns of autofluorescence in both eyes characterized by concentric rings of alternating hyper- and hypoautofluorescence, and foveal sparing of photoreceptors and RPE, as seen on SD-OCT, bilaterally. After negative results in screening for mutations in candidate genes including ABCA4

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and PRPH2, DNA from 3 members of the family, including both affected siblings and their mother, was screened by whole exome sequencing resulting in identification of 2 *CRB1* missense mutations, c.C3991T:p.R1331C and c.C4142T:p.P1381L, which segregated with the disease in the family. Of the 2, the p.R1331C *CRB1* mutation has not been described before and the p.P1381L variant has been described in 1 patient with Leber congenital amaurosis.

Conclusions—This report illustrates a novel presentation of a macular dystrophy caused by *CRB1* mutations. Both affected siblings exhibited a relatively well-developed retinal structure and preservation of generalized retinal function. An unusual 5-year progression of macular atrophy alone was observed that has not been described in any other *CRB1*-associated phenotypes.

Mutations in the *CRB1* gene (Mendelian Inheritance in Man #604210) have been associated with a variety of generalized retinal dystrophies ranging from retinitis pigmentosa (RP) to Leber congenital amaurosis (LCA).¹⁻⁴ Retinitis pigmentosa refers to a group of clinically and genetically heterogeneous disorders affecting 1.5 million people worldwide. Reported cases of RP associated with mutations in *CRB1* (RP12 phenotype) present with an early disease onset, including nystagmus, hyperopia, optic nerve head drusen, relative attenuation of the vessels, a maculopathy, and nummular type of pigmentation in the periphery.^{2,5,6} *CRB1* mutations also have been correlated to retinal vascular sheathing, preserved para-arteriolar retinal pigment epithelium (RPE), and the development of Coats-like exudative vasculopathy, a condition of abnormally permeable blood vessels leading to exudation and retinal detachment.^{4,5,7} Mutations in the *CRB1* gene have been detected in 10% to 13% of patients with LCA, one of the most severe forms of retinal dystrophy characterized by onset in the first year of life, nystagmus, sluggish pupillary and oculodigital reflexes, and an extinguished electroretinogram (ERG).^{4,8-10} Phenotypically, patients with LCA with *CRB1* mutations usually show the described RP12 characteristics, including the early-onset maculopathy (macular dysplasia).

CRB1 is the human homologue of the gene encoding the crumbs (Crb) protein in *Drosophila melanogaster* and is expressed in the fetal brain and the inner segments of photoreceptors in humans.^{2,11} *CRB1* also is expressed in the brain, kidney, colon, stomach, lung, and testis.^{12,13} *CRB1* maps to chromosome 1q31.3 and is composed of 12 exons that are translated into 2 protein isoforms, the larger of which possesses a cytoplasmic domain containing FERM- and PDZ-binding motifs that enable adherin junction formation and actin skeleton association.¹⁴ *CRB1* is crucial for the assembly of the zonula adherens in *Drosophila* and has been found to be localized at the apical membrane.^{15,16} A similar distribution in the outer limiting membranes of epithelial cells, Müller cells, and photoreceptor inner segments has been observed in mouse and human retinas.^{11,17,18} Developmentally, *CRB1* has been shown to determine embryonic epithelium and peripheral neurons in *Drosophila*.^{15,16} In addition, both human and mouse *CRB1* proteins are involved in photoreceptor morphogenesis.^{11,17,19} Mouse models of *CRB1* have been extensively studied and used to show developmental defects and disorganization of the retina in mutants, particularly disruptions of the outer limiting membrane and the formation of retinal folds or pseudorosettes.^{17,19} These findings correlate with the appearance of the developmentally immature retinas in patients with *CRB1* mutations. The retinas of such patients appear

thickened and often exhibit altered laminar organization through the disruption of developmental apoptosis.^{19-21,28}

More than 150 disease-associated variants have been described to date in the *CRBI* gene, the most common of which is the p.C948Y variant in exon 9.^{1,2,5,11,20,22-25} We describe the clinical appearance of a combination of novel *CRBI* variants that were associated with an unusual and previously not described phenotype in 2 affected siblings of Irish descent.

Methods

Patients and Clinical Evaluation

Two patients, the proband and her affected brother, along with an unaffected sister, mother, and father, were enrolled in the study under the protocol #AAAB6560 after obtaining full consent. The protocol was approved by the institutional review board at Columbia University and adhered to tenets set out in the Declaration of Helsinki.

Each patient underwent a complete ophthalmic examination by a retinal physician (S.H.T.), which included color fundus photography with an FF 450plus Fundus Camera (Carl Zeiss Meditec AG, Jena, Germany). Fundus autofluorescence (FAF) images were obtained using a confocal scanning-laser ophthalmoscope (Heidelberg Retina Angiograph 2, Heidelberg Engineering, Dossenheim, Germany) by illuminating the fundus with argon laser light (488 nm) and viewing the resultant fluorescence through a band pass filter with a short wavelength cutoff at 495 nm. Simultaneous FAF and spectral domain optical coherence tomography (SD-OCT) images were acquired using a Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany). Color-coded retinal thickness maps were exported from Heidelberg Explorer v. 5.4.6.0 (Heidelberg Engineering, Heidelberg, Germany) software that had automatically calculated the thickness of the retina (from internal limiting membrane to Bruch's membrane) using raster SD-OCT scans acquired on the Heidelberg Spectralis. The segmentations performed by the software were manually adjusted where necessary. The retinal thickness measurements are automatically mapped onto an infrared fundus image and color-coded according to thickness (micrograms). Electroretinography was carried out using the Diagnosys Espion Electrophysiology System (Diagnosys LLC, Littleton, MA). For all recordings, the pupils were maximally dilated before full-field ERG testing using guttate tropicamide (1%) and phenylephrine hydrochloride (2.5%), and the corneas were anesthetized with guttate proparacaine 0.5%. Silver-impregnated fiber electrodes (DTL; Diagnosys LLC) were used with a ground electrode on the forehead. Full-field ERGs to test generalized retinal function were performed using extended testing protocols incorporating the International Society for Clinical Electrophysiology of Vision standard.²⁶ Microperimetry (Nidek Instruments Inc., Padova, Italy; NAVIS software version 1.7.3.) mapping was carried out in proband using the 10-2 pattern after pupil dilation with 1% tropicamide and after a 15-minute adaptation period to the background luminance.

Genetic Analyses

The proband was initially screened for variants in the *ABCA4* and *PRPH2* genes by direct Sanger sequencing revealing no disease-associated variants. Because the phenotype did not

suggest testing any other candidate genes, the family was subjected to the whole exome sequencing and analysis.

Exome sequencing was performed for the 2 affected siblings and their unaffected mother. Three to 5 μ m of genomic DNA extracted from peripheral blood were exome captured and sequenced at Axseq Technologies (Rockville, MD; available at: www.axeq.com; accessed September 10, 2013). In-solution sequence capture was performed using Nimblegen capture array (SeqCap EZ Exome Library v3.0) with 64 Mb target region. Massively parallel sequencing of the enriched library was performed on Illumina HiSeq platform with 100 base pair paired-end reads. Sequencing reads were generated in the fastq format after nucleotide calling, and quality score assessment was performed using instrument-specific Real Time Analysis software (Illumina, San Diego, CA). Read pairs were aligned to the human reference genome (hg19) using Burrows-Wheeler Aligner (available at: <http://bio-bwa.sourceforge.net/>), and duplicate reads were removed with PICARD tools (available at: <http://picard.sourceforge.net/>). Uniquely mapped on-target reads were extracted, and single nucleotide polymorphism (SNP) and in/del calling were performed with Samtools (<http://samtools.sourceforge.net/>). Variants were annotated using ANNOVAR (<http://www.openbioinformatics.org/annovar/>). All variants of interest were confirmed by Sanger sequencing, and segregation analyses were performed in the entire family.

Results

Clinical Examination

The proband, a 45-year-old woman of Irish descent, had noticed a decrease in her vision starting around her mid-20s. Her brother, aged 41 years at the time of examination, had reported similar onset of visual symptoms in his early 30s. Neither the proband nor the affected sibling had undergone ophthalmic examination before the onset of symptoms; therefore, there are no data on the pre-symptomatic retinal state in either case.

The third sibling and parents reported no major issues with vision (Fig 1). The family members recalled a significant vision loss in their maternal grandmother and a paternal aunt beginning in the 6th and 7th decades of life. However, in both cases the cause remained unknown, and both of these individuals were deceased with no available clinical records. Both the proband and her affected sibling did not have any contributory ophthalmic or systemic illnesses, and both denied a history of smoking.

Table 1 summarizes the demographic and clinical findings from the initial evaluation of both affected siblings. Best-corrected visual acuity in the proband was 20/40 in the right eye and 20/400 in the left eye at presentation. A slit-lamp examination of the anterior segment showed unremarkable results. Dilated funduscopy revealed a clear vitreous and healthy vascular arcades. There was no optic disc swelling; however, temporal pallor was present in both eyes (Fig 2A, F). There were no signs of macular edema in the right eye, although both maculae exhibited a mottled, granularly speckled appearance with a continuous annulus of atrophy circumscribing an island of preserved RPE in the foveal region. A more generalized “wipe-out” of RPE in the left macula was observed. No peripapillary sparing was noted in either eye, and, most notable, atrophy of the retina and RPE was observed nasal to the disc

in both eyes. Central fixation was spared according to the patient's ability to follow a fixation target (Fig 2A, F).

The younger brother of the proband presented with similar ocular findings. His best-corrected visual acuity was 20/40 in the right eye and 20/70 in the left eye. Cystoid macular edema (CME) was present in the left eye that responded to treatment with oral acetazolamide (500 mg) and guttate nepafenac, resulting in the improvement of visual acuity to 20/30 with an associated reduction in the size of intraretinal cysts. The anterior segment examination results were unremarkable. No vitreous opacities were found, and both the optic nerves and the fundus vasculature appeared healthy. Both maculae exhibited similar granular characteristics observed in the proband; however, clear parafoveal atrophy was seen in the maculae (right eye > left eye) along with a few foci of hyper-pigmentation (Fig 2L, Q).

Both parents of the affected siblings were examined with dilated fundus examination. There was no vascular attenuation or intraretinal pigment migration in either parent. However, peripapillary atrophy was present in both parents. Choroidal thinning due to myopia was observed in the mother's left eye. A nonsignificant epiretinal membrane was observed in the temporal macula of her left eye. Normal retinal layers and intact photoreceptors were apparent in both parents (Fig 3B, D, F, H).

Fundus Autofluorescence

Fundus autofluorescence imaging in the proband showed hyperautofluorescence suggestive of preservation of a central island of RPE in the right eye and parafoveal preservation of an RPE island in the left eye. The macula in both eyes appeared to be enshrouded with a large cloud of hypoautofluorescence extending temporally past the optic discs. Peripheral areas of relatively normal FAF pattern were marked with dark punctate changes in areas approaching the hypoautofluorescent cloud (Fig 2B, C, G, H).

In the affected brother, the central maculae of both eyes appeared hypoautofluorescent, more so in the left eye. There was associated hypoautofluorescence along the peripapillary area and the proximal superotemporal arcades in both eyes. However, the more peripheral maculae appeared relatively hyperautofluorescent compared with the surrounding extramacular retina. There were multiple discrete punctate hyperautofluorescent foci in the maculae, predominating nasally and temporally (Fig 2N, S). A petaloid pattern of alternating hyper- and hypoautofluorescence was seen in both eyes but was, again, more obvious in the left eye (Fig 2S).

Spectral Domain Optical Coherence Tomography

Horizontal SD-OCT line scans through the central maculae of both eyes of the proband showed an absence of the outer nuclear layer, inner segment ellipsoid band, or inner/outer segment junction of the photoreceptors and RPE, except in the foveal region of the right eye where the structure of both the photoreceptors and RPE appeared anomalous, but present, suggestive of relative sparing (Fig 2D, E). This sparing co-localized with the region of hyperautofluorescence. Of note, similar sparing of the photoreceptors was not seen in the foveal region of the left eye despite a hyperautofluorescent signal (Fig 2I, J). The SD-OCT

scans in the right eye of the affected sibling resembled that of the proband. However, the photoreceptor sparing on SD-OCT was less obvious. Nonetheless, in the foveal region of the sibling's right eye there was evidence of a residual inner segment ellipsoid band in the foveal region and a disorganized atrophic outer nuclear layer. On close inspection of the outer nuclear layer in the temporal parafoveal macula of the right eye, there was possible early cystoid change (Fig 2O). There was definite CME in the left eye visible on SD-OCT (Fig 2T, U). Despite the presence of CME in the left eye, the outer nuclear layer and inner segment ellipsoid band appeared well preserved in the central macula. A 3-year followup scan of the affected sibling's left eye was acquired after treatment (Fig 4, available at www.aaojournal.org) and showed that there was persistence of CME, although it had reduced. Even with progressive photoreceptor and RPE layer disruption, retinal thickness appeared to be within normal limits in both affected siblings (excluding the sibling's left eye with CME), with reasonably well-preserved retinal lamination.

Retinal thickness in the proband and affected brother was assessed using color-coded thickness maps over the macula of each eye (Fig 5). Compared with an age-matched control, both eyes of the proband and the right eye of her affected sibling exhibited a reduction in retinal thickness in the central macula consistent with atrophy. There was a trend toward increased thickness relative to the control in the more peripheral macula. The left eye of the proband's sibling had grossly increased macular thickness centrally due to CME.

Electroretinogram Analysis

Scotopic responses in the proband showed a generalized preservation of retinal rod function. A marginal implicit time delay was detected in the photopic responses, suggestive of cone involvement; however, both the waveform and amplitudes were within normal limits compared with age-matched controls (Fig 2K).

Progression and Visual Function

Microperimetry 1 (MP-1) mapping results (in decibels) were recorded in the proband at the initial visit and registered to a corresponding FAF image with the built-in Navis software. Preserved visual function was documented within the region of hyperautofluorescence in both eyes. However, across the atrophic hypoautofluorescent macula, retinal sensitivities of 0 decibels were recorded. The test documented steady foveal fixation as assessed by the fixation tracker on the mapping instrument. A subsequent recording after 5 years showed a notable reduction in sensitivity in the right eye and an almost complete loss of function in the left eye (Fig 6).

Genetic Analyses

Whole exome sequencing was performed in the 2 affected siblings and their unaffected mother. An average of 113 million total reads were generated for each sample, with an average of 65 million (58%) nonredundant, unique reads mapped to the 64 MB exome region (Table 2) (available at www.aaojournal.org). More than 90% of the target regions have $>10\times$ coverage, with an average mean depth of coverage of $80\times$. On average, there were a total of 99,041 variants identified for each sample, of which 20 730 (21%) were in protein coding sequences. Some 98% of all coding variants were SNPs and 2% were

insertions and deletions (in/dels). The ratio of nonsynonymous to synonymous variants was close to 1:1 in all samples. For initial filtering of variants, the minimum SNP quality value was set at 20 and the minimum total read depth was set at 5.

Considering the autosomal recessive inheritance pattern of the disease and that both affected siblings had to have the same causal mutations, we focused on genes that had at least 2 shared variants in the 2 affected siblings (Table 3). We further assumed that the disease-causing variants have to be rare, thereby filtering the sequence data for new variants and those found in <0.5% in dbSNP135 and in the 1000 Genomes databases. After excluding synonymous variants and all coding and intronic variants that did not affect splicing, the number of candidate genes was narrowed down to 19. After assessing the phase of remaining variants using the sequences of the mother, only 3 possible candidate genes remained: *CRB1*, *NADK*, and *DNAH12*. The segregation of variants in these genes with the disease was tested in the entire family, including the unaffected sibling and the father.

Dynein, axonemal, heavy chain 12 protein is a large protein with multiple rare variants involved in microtubule-associated motor protein complexes composed of several heavy, light, and intermediate chains. *NADK* catalyzes the synthesis of NADP from NAD and ATP and exists in several isoforms. Neither of these 2 genes has been associated with eye disease phenotypes, and there is no direct mechanism how these would be involved in retinal dystrophies.

This left *CRB1* as the only plausible candidate gene for the disease phenotype segregating with the 2 variants, because it is a well-known gene in retinal dystrophies (RetNet; <https://sph.uth.edu/retnet/>; accessed September 10, 2013). The 2 *CRB1* variants shared by the affected siblings (c.C3991T:p.R1331C and C4142T:p.P1381L) are not present in ESP6500, dbSNP135, and 1000 Genomes database, although the p.P1381L variant was recently identified as disease associated in 1 patient with LCA.²⁷ Both variants are predicted to be deleterious or damaging by SIFT, Polyphen-2, and MutationTaster programs. Nucleotide positions of the 2 mutations are highly conserved as predicted by phyloP. The novel c.C3991T variant that results in the p.R1331C mutation is 1 nucleotide before a previously reported benign variant c.A3992G, which results in the p.R1331H amino acid change. The latter is considered benign; however, unlike arginine to histidine change (Grantham distance 29), there is large physicochemical difference between arginine and cysteine (Grantham distance 180). Creation of a cysteine residue at this position also results in 2 consecutive cysteines in the amino acid chain. Because EGF-like domain is characterized by 6 conserved cysteines forming 3 disulfide bonds and modification from arginine to histidine at this position is considered benign, p.R1331C also could be a relatively milder mutation, which could explain the milder phenotype compared with other *CRB1*-associated phenotypes.

Discussion

The clinical and genetic findings were summarized in a family in whom 2 rare, likely disease-associated *CRB1* missense variants segregated with an unusual macular dystrophy phenotype. Previously reported phenotypes associated with mutations in *CRB1* include those consistent with an early-onset RP, associated with preserved para-arteriolar RPE or Coats-

like exudative vasculopathy. The patients described in this study exhibited unusual phenotypic characteristics that have not been described before and therefore eluded clinical diagnosis by several retinal specialists. Both affected siblings presented with decreased visual acuity but without nyctalopia. Furthermore, on fundoscopy, there was a relatively normal-appearing periphery without any of the RP12 characteristics, such as nummular hyperpigmentation and some degree of vascular attenuation. The disease was largely confined to the macula. In both cases, the reported age of onset was between the mid-20s and early 30s; however, the proband had progressed to a more advanced disease stage as evidenced by her comparatively lower visual acuity and the extent of macular atrophy on FAF imaging and SD-OCT. Of note, a small patch of RPE was preserved in the central macular regions, and the overlying retina was found to be functionally intact through microperimetry testing in the proband. Spectral domain OCT documented relative foveal sparing of the photoreceptors in the right eye of the proband and in both eyes of the affected sibling, although the latter also had CME in his left eye. Although the proband did not exhibit any CME at the time of examination, retinal laminar changes seen in her foveal SD-OCT scans may be due to macular edema from an earlier disease stage.

Hyperautofluorescence in the nasal retina and foveal sparing are rare phenomena observed in some cases of ABCA4- and RDS-associated maculopathies. Clinical findings in the affected brother exhibited unusual alternating patterns of autofluorescent rings marked with punctate changes in the central macula, resembling a bull's-eye lesion reported in other related retinal conditions.

Patients with *CRB1*-associated phenotypes typically present with a thick, underdeveloped retina characteristically exhibiting loss of laminar layering. A study of mice carrying the rd8 mutation in *Crb1* has shown an analogous phenotype of a thickened retina and loss of distinct retinal layering.²⁸ This developmental disorganization has been attributed to the role of *CRB1* in embryonic retinal development in mice and humans, more specifically, photoreceptor morphogenesis. Abnormal thickening and loss of laminar layering were not seen in the presented cases. Although some laminar disruption is evident, the distinct layers can, in general, be distinguished and retinal thickness appears normal. Preserved electrophysiologic function was observed in full-field ERG measurements. These findings are consistent with the presence of peripheral retinal sparing but distinct from previous reports of *CRB1*-associated phenotypes in which ERG was extinguished and undetectable because of abnormal development of the embryonic retina.

The molecular genetic reasons for the observed distinct phenotype may be attributed to a specific combination of *CRB1* alleles, to modifier genes, or both. Furthermore, the modifying effect of nongenetic factors (e.g., environmental) has been suggested as a reason for phenotype variation in *CRB1* retinopathy.²³ As discussed previously, the combination of the alleles, specifically the new p.R1331C variant, may have a different effect on the protein function than other *CRB1* alleles. The exome sequences also were analyzed for variants in possible modifier genes, especially those that have been shown to interact with the *CRB1* protein or belong to the same pathway(s) (Table 4, available at www.aaojournal.org). Specifically, genes that encode for proteins involved in the CRUMBS network, those that

have been co-immunoprecipitated with *CRB1* or retinal ciliopathy proteins in the ciliary compartment, were analyzed for possible modifier variants (Table 5).

Crb1 in mice localizes to the outer limiting membrane between the subapical surface or region and adherens junction of Müller glia cells.¹⁹ In the outer limiting membrane, *Crb1* interacts with MPP5 via its PDZ binding domain and EPB41L5 with its FERM binding site. At this location, MPP5 organizes a protein scaffold that includes the MAGUK family members MPP3 and MPP4. In addition, MPP5 also has been found to interact with LIN-7, PAR6, PATJ, MUPP1, EZRIN, and the neuronal GABA transporter GAT1.¹⁴ No mutations that were shared between both affected siblings were found, except for a rare heterozygous missense mutation in *MPDZ*, a gene that codes for multi-PDZ domain protein-1 (MUPP1), in the proband (Table 5); MUPP1 interacts with the intracellular domain of *CRB1* via association with the PDZ domain of MPP5.

Some rare heterozygous variants in genes known to cause retinal dystrophies, such as RP, LCA, and CRD, were shared by both affected siblings. The specific variants and in silico prediction of their pathogenicity are shown in Table 5. One study has suggested that the USHERIN protein network has physical connection to the CRUMBS protein complex via interaction of MPP5 with MPP1 and the multi-PDZ protein whirlin at the outer limiting membrane.¹⁴ *WHRN* and *USH2A* co-localize at the outer limiting membrane and the connecting cilium of photoreceptors.²⁹ Mutations in *USH2A* are associated with recessive Usher syndrome type 2a and recessive RP. A study of Spanish families with LCA has suggested that variants in *RPGRIP1*, along with *GUCY2D* and *AIPL1*, could be modifier alleles of *CRB1*.³⁰ Several studies have identified mutations in the *TOPORS* gene that cause dominant RP.^{31,32} *TOPORS* is a cilia-centrosomal protein that localizes to the basal bodies of connecting cilium and to the centrosomes of cultured cells. Morpholino-mediated silencing of topors in zebrafish embryos demonstrated defective retinal development and failure to form outer segments.³³ *CDHR1* (*PCDH21*) encodes a photoreceptor-specific cadherin that co-localizes at the base of outer segment with Prominin 1. It is involved in disc morphogenesis and causes cone-rod dystrophy in a mutated form.³⁴ In addition, sequence changes in *CEP290* and *SNRNP200* were detected in the proband and 1 variant in the *c2orf71* gene in the affected brother. Recessive mutations in all these genes have been associated with LCA, RP, or CRD. However, because both patients exhibited similar disease phenotype and the combination of *CRB1* variants was unique to this family, we were not able to assign modifier role to any of the identified variants or to nongenetic modifiers.

In conclusion, manifestation of only focal disease in *CRB1*-associated degeneration in this family was distinct from all previously described retinal dysfunction caused by mutations in *CRB1*. Instead of a generalized retinal degeneration with dysplastic retinae seen in other *CRB1*-associated cases, patients in this study exhibited a slowly progressive focal disease. Patients also were lacking all well-known phenotypic features of *CRB1*-associated disease, such as peripheral nummular pigmentation, preserved para-arteriolar RPE, or Coats-like vasculopathy. Although no unequivocal evidence was found for any modifier alleles that could explain the clinical findings, variants in several genes were identified that could modulate the phenotype in this family. Identification of gene- and especially mutation-

specific phenotypes will aid in directing future DNA testing and selecting treatment options for patients with macular dystrophies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations and Acronyms

CME	cystoid macular edema
ERG	electroretinogram
FAF	fundus autofluorescence
LCA	Leber congenital amaurosis
MP-1	microperimetry 1
MUPP1	multi-PDZ domain protein-1
RP	retinitis pigmentosa
RPE	retinal pigment epithelium
SD-OCT	spectral domain optical coherence tomography
SNP	single nucleotide polymorphism

Figure 1.

Pedigree of the family. *Open circles* and *squares* represent the unaffected female and male family members, respectively; *closed circles* and *squares* represent the affected female and male patients. The mother and father in this pedigree are heterozygous for the p.P1381L and p. R1331C mutations, respectively. The affected siblings are compound heterozygous for both mutations.

Figure 2.

Fundus photographs, fundus autofluorescence (FAF) images, and spectral-domain optical coherence tomography (SD-OCT) scans of the right and left eyes of a 45-year-old woman (proband) and her 41-year-old affected brother with the same *CRB1* mutations. Fundus photography in the proband exhibited stable fixation (needle position), temporal pallor of the optic discs, attenuated retinal vasculature, and extensive retinal pigment epithelium (RPE) changes (A, F). The FAF imaging reveals predominant hypoautofluorescence across the macula, consistent with widespread RPE atrophy, although relative hyperautofluorescence was documented in the foveal and parafoveal regions of the right and left eyes, respectively, consistent with sparing of the RPE (B, C, G, H). The SD-OCT scans reveal relative sparing of foveal photoreceptors (outer nuclear layer, inner segment ellipsoid band) and RPE in the right eye (D, E), and an absence of all except RPE in the left eye (I, J). Fundus photographs in the affected brother also showed extensive loss of macular RPE (L, Q). The FAF revealed concentric rings of alternating hyper- and hypoautofluorescence encircling the macula (M, R), and there were petaloid patterns centrally, greatest in the left eye (N, S). The SD-OCT scans through the fovea in the right eye revealed an area of relative foveal sparing (O, P), and cystoid macular edema (CME) was detected in the left eye (T, U). There was possible early cystoid change in the temporal parafoveal macula of the right eye (O). Relatively normal laminar architecture is preserved in the maculae of both affected siblings. Electrophysiology in the proband reveals overall preservation of generalized cone and rod function without any obvious implicit time shifts (K). ISe = inner segment ellipsoid; OD = right eye; ONL = outer nuclear layer; OS = left eye.

Figure 3.

Fundus photographs, 488 nm autofluorescence (AF) images and spectral-domain optical coherence tomography (SD-OCT) scans in the parental Q5 carriers of the *CRB1* mutation. No significant retinal pigment epithelium (RPE) and abnormal AF patterns were observed. Both parents exhibited peripapillary atrophy in both eyes, greatest in the mother and related to her high myopia. Minor choroidal thinning in the mother also was attributed to this myopia (**B, D**). The SD-OCT of her left eye revealed a nonsignificant epiretinal membrane. Otherwise, normal retinal layers and intact photoreceptors were present in both parents. FAF = fundus autofluorescence; OD = right eye; OS = left eye.

Figure 5.

Color-coded macular thickness (μm) maps of the *CRB1*-affected proband and her brother compared with an age-matched normal subject. The maps show a reduced (darker colors) thickness in the central macula in both eyes of the proband and in the right eye of her sibling compared with the control, with a trend toward increased (brighter colors) thickness relative to the control in the more peripheral macula. The left eye of the proband's sibling had grossly increased macular thickness due to cystoid macular edema (CME).

Figure 6.

Functional and macular progression assessment through fundus autofluorescence (FAF) imaging and microperimetry (MP)-1 mapping. Over the course of 5 years, preservation of the central island of hyperautofluorescent retinal pigment epithelium (RPE) was maintained; however, the spatial function had become more constricted. A comparative overlay of MP-1 and FAF showed that the scotomata (0 decibels) recorded on the MP-1 corresponded to atrophic (hypoautofluorescent) regions. OD = eye; OS = left eye.

Table 1

Summary of Demographic, Clinical, and Genetic Data

Family	Age (yrs)	BCVA Snellen (logMAR) OD	BCVA Snellen (logMAR) OS	Condition	Age of Onset	CRB1 Mutation
Proband	45	20/40	20/400	Affected	Mid-20s	R1331C; P1381L
Brother	41	20/40	20/70	Affected	Early 30s	R1331C; P1381L
Sister	33	-	-	Unaffected*	-	wt; wt
Mother	69	20/50	20/40	Unaffected	-	wt; P1381L
Father	85	20/20	20/20	Unaffected	-	R1331C; wt

BCVA = best-corrected visual acuity; logMAR = logarithm of the minimum angle of resolution; OD = right eye; OS = left eye.

* Not clinically examined; underwent only genetic testing.

Table 3
Variants Identified in the 2 Affected Individuals

Variant Filters	No. SNVs	No. Genes
Total variants shared between both affected siblings	67,951	20,181
Not present in dbSNP135 common and <0.5% in 1000 genomes	5733	4499
Missense, nonsense, indels, and splicing	392	364
Recessive	47	19
Phase-assessed	6	3

SNV = single nucleotide variation.

Table 5

Potential Modifier Genes for CRR1-Associated Phenotype

Gene	DNA Change	Protein Change	PP-2	SIFT	Mutation Taster	PhyloP	In Proband/Brother
MPDZ	C1970T	P657L	Benign	Deleterious	Disease causing	Weakly conserved	Proband
USH2A	A10999C	T3667P	N/A	Deleterious	Polymorphism	Not conserved	Both
RPGRIPI	C2794G	P932A	Probably damaging	Tolerated	Disease causing	Moderately conserved	Both
TOFORS	G2138A	R713K	Benign	Tolerated	Polymorphism	Moderately conserved	Both
CDHR1	A1868G	N623S	Benign	Tolerated	Polymorphism	Highly conserved	Both
CEP290	G4237C	D1413H	Benign	Deleterious	Disease causing	Moderately conserved	Proband
SNRNP200	A3315G	A1105A	Synonymous	Synonymous	Synonymous	Synonymous	Proband
c2orf71	G3789A	L1263L	Synonymous	Synonymous	Synonymous	Synonymous	Brother

N/A = not applicable.