Biallelic Mutations in CRB1 Underlie Autosomal Recessive Familial Foveal Retinoschisis

Ajoy Vincent,1–3 Judith Ng,1 Christina Gerth-Kahlert,4 Erika Tavares,1 Jason T. Maynes,5 Thomas Wright,2 Amit Tiwari,6 Anupreet Tumber,2 Shuning Li,1 James V. M. Hanson,4 Angela Bahr,6 Heather MacDonald,2,7,8 Luzy Bähr,6 Carol Westall,2,3 Wolfgang Berger,6,9,10 Frans P. M. Cremers,11 Anneke I. den Hollander,11,12 and Elise Héon1–3

1Program of Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Canada
2Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, University of Toronto, Toronto, Canada
3Department of Ophthalmology and Vision Sciences, University of Toronto, Toronto, Canada
4Department of Ophthalmology, University Hospital Zurich, Zurich, Switzerland
5Department of Anesthesia and Pain Medicine, and Program in Molecular Structure and Function, The Hospital for Sick Children, Toronto, Canada
6Institute of Medical Molecular Genetics, University of Zürich, Wagistrasse 12, Schlieren, Switzerland
7Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada
8Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada
9Zurich Center for Integrative Human Physiology (ZIHP), University of Zürich, Zurich, Switzerland
10Neuroscience Center Zurich (ZNZ), University and ETH Zürich, Zurich, Switzerland
11Department of Human Genetics, Radboud University Medical Centre, Nijmegen, The Netherlands
12Department of Ophthalmology, Radboud University Medical Centre, Nijmegen, The Netherlands

Correspondence: Elise Héon, Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada; elise.heon@sickkids.ca.
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PURPOSE. To identify the genetic cause of autosomal recessive familial foveal retinoschisis (FFR).

METHODS. A female sibship with FFR was identified (Family-A; 17 and 16 years, respectively); panel based genetic sequencing (132 genes) and comparative genome hybridization (142 genes) were performed. Whole-exome sequencing (WES) was performed on both siblings using the Illumina-HiSeq-2500 platform. A sporadic male (Family-B; 35 years) with FFR underwent WES using Illumina NextSeq500. All three affected subjects underwent detailed ophthalmologic evaluation including fundus photography, autofluorescence imaging, spectral-domain optical coherence tomography (SD-OCT), and full-field electroretinogram (ERG).

RESULTS. Panel-based genetic testing identified two presumed disease causing variants in CRB1 (p.Gly123Cys and p.Cys948Tyr) in Family-A sibship; no deletion or duplication was detected. WES analysis in the sibship identified nine genes with two or more shared nonsynonymous rare coding sequence variants; CRB1 remained a strong candidate gene, and CRB1 variants segregated with the disease. Wes in Family-B identified two presumed disease causing variants in CRB1 (p.Ile167_Gly169del and p.Arg764Cys) that segregated with the disease phenotype. Distance visual acuity was 20/40 or better in all three affected except for the left eye of the older subject (Family-B), which showed macular atrophy. Fundus evaluation showed spoke-wheel appearance at the macula in five eyes. The SD-OCT showed macular schitic changes in inner and outer nuclear layers in all cases. The ERG responses were normal in all subjects.

CONCLUSIONS. This is the first report to implicate CRB1 as the underlying cause of FFR. This phenotype forms the mildest end of the spectrum of CRB1-related diseases.

Keywords: retinoschisis, macular degeneration, macular edema, familial foveal retinoschisis, human CRB1 protein, optical coherence tomography, electroretinogam (ERG), electroretinography

Familial foveal retinoschisis (FFR) is an extremely rare autosomal recessive disorder first described by Lewis et al., in 1977.1 Affected cases present within the first two decades of life with reduced distance visual acuity, usually in the range of 20/30 to 20/60. The retina shows the characteristic fovea centered cart-wheel lesion restricted to the macula; the peripheral retina is normal.1–3 The cart-wheel lesions reflect schitic or cystoid changes, clearly evident on optical coherence tomography (OCT). Mild protan or tritan color vision anomalies may be observed. Full-field ERG is usually normal in keeping with the maculopathy; however, the dim light scotopic ERG can be subnormal.1 In 2003, Kabanarou et al.2 introduced the term isolated foveal retinoschisis to accommodate both sporadic and familial cases...
of the disorder. There is high female preponderance as all nine cases reported in literature are females; the reason for this is unknown.4,5 (Lorenz B, et al. IOVS 2000;41:ARVO Abstract S885).

In humans, CRB1 is expressed in the retina and brain, and codes for the human orthologue of Drosophila melanogaster transmembrane protein Crumbs.4 Alternate splicing leads to two isoforms, 1376 amino acids (AF_154671) and 1406 amino acids (NP_957705) in length.4,5 Both isoforms have extracellular and cytoplasmic domains; but the larger transcript also has transmembrane domain formation. The Cys948Tyr mutation disrupts a disulfide bridge, which are important for internal structural rigidity of the domain (disulfide shown by #). The caret (^) is the position of the residue 168, showing how a deletion would affect the beta-turn of the EGF-like domain.

Clinical Evaluation

Four members in Family-A (proband [II-1], affected sibling [I-2], and unaffected parents) and proband from Family-B (II-2) underwent detailed eye examination including best corrected visual acuity (BCVA), fundus photography, fundus autofluorescence testing (FA: Visucam500 from Carl Zeiss Meditec, Jena, Germany; or Spectralis, Heidelberg Engineering, Heidelberg, Baden-Württemberg, Germany) and spectral-domain OCT (SD-OCT; Cirrus; Carl Zeiss Meditec or Spectralis, Heidelberg Engineering).

Full-field ERG testing incorporating International Standards1,2,3 and intravenous fluorescein angiography were performed on all three affected individuals. Goldmann Visual Fields (GVF) testing using both I4e and III4e isopters were performed on all three affected individuals. Goldmann Visual Fields (GVF) testing using both I4e and III4e isopters were performed in affected individuals in Family-A.

Segmentation analysis was performed on the SD-OCT from the left eye in all three affected individuals. In Family-A, a 6 × 6 mm region of retina centered on the fovea was scanned using the 512 × 128 macular cube protocol using Cirrus HD-OCT 5000. Control data was collected from one eye of 40 patients with normal visual development (median age: 23.5 years, range, 12–35 years), recruited as part of a separate study. Ten retinal layers were identified in each macular cube OCT using Iowa Reference Algorithms (Retinal Image Analysis Lab, Iowa Institute for Biomedical Imaging, Iowa City, IA, USA).14,15 Custom software averaged thickness measurements over retinal regions corresponding to the three rings suggested by the Early Treatment Diabetic Retinopathy Study (ETDRS)17; the ETDRS ring design consists of a central foveal region with a 1-mm diameter, an inner ring with and inner and outer diameter of 1 and 3 mm, respectively, and an outer ring with inner and outer diameters of 3 and 6 mm, respectively. The OCT in family B was performed using Spectralis; a 30° (~8.3 × 8.3 mm) square centered on the fovea was scanned using the 1536 ×
496 line protocol. The ETDRS regions were segmented and averaged using the built-in software on the system. The total retinal thickness and thickness of ganglion cell-inner plexiform complex (GCL-IPL; as it contained the least amount of schitic changes) were measured.

Genetic Testing

**Family-A.** Multistep clinical genetic testing was performed. In total, 132 genes known to cause retinal dystrophy were screened using PCR amplification and next-generation or Sanger sequencing (Supplementary Table S1). A comparative genomic hybridization technique was used to test for deletion or duplication in 142 genes associated with non-syndromic/syndromic inherited eye dystrophies (Supplementary Table S1). Whole exome capture and sequencing (WES) was performed in the two affected siblings at The Center for Applied Genomics (TCAG), Toronto, Canada and analyzed using standard pipeline (Supplementary Methods). Overall mean target exon coverage was 101× and 110× in II-1 and II-2, respectively; 95% of targets in both siblings had a greater than 20× base coverage. The potential effect of rare (allele frequency ≤ 0.01) coding-sequence single nucleotide variants (SNVs) were predicted using six predictive tools that included Polyphen-2, SIFT, mutation assessor (Ma), combined (SNVs) were predicted using six predictive tools that included Polyphen-2, SIFT, mutation assessor (Ma), combined (SNVs) were predicted using six predictive tools that included Polyphen-2, SIFT, mutation assessor (Ma), 21 combined annotation-dependent depletion (CADD) Phred, 22 and conservation values amongst PhyloP placental mammals and PhyloP 100-vertebrates. A cut off score was set for each tool (Polyphen-2 ≥ 0.95; SIFT ≤ 0.05, Ma ≥ 2.0, CADD Phred ≥ 15, Average PhyloP nucleotide conservation inferred from placental mammal: value ≥ 1.0 and Average PhyloP nucleotide conservation inferred from 100 vertebrates: value ≥ 1.0). Any SNV that met the cut off for a specific predictive tool was given a score of 1; a maximum cumulative score of 6 was possible for any SNV. If a SNV did not meet the cut off for a specific tool, then a score of 0 was given.

**Family-B.** Genomic DNA was isolated from patients’ blood sample and WES was performed using standard protocols (Supplementary methods). A filtering approach was established to exclude known single nucleotide polymorphisms or benign sequence variations. Mutations that have been previously described to be disease causing in the Human Gene Mutation Database and literature were given the highest priority followed by protein truncation mutations (nonsense and frameshift variants). Sequence variants considered relevant for the disease, were confirmed by conventional Sanger sequencing.

**RESULTS**

**Clinical Phenotype: Family-A**

**Proband (II-1).** A 17-year-old female born to nonconsanguineous Caucasian parents (Fig. 1A) presented with a 4-year history of diminution in distance vision. There is no history suggestive of photophobia or nyctalopia. The BCVA was 20/30 and 20/40 in the right and left eyes, respectively. Color vision was normal in both red-green and blue-yellow axes. The contrast sensitivity was 1.35 log units in each eye. Fundus evaluation showed spoke-wheel appearance at the fovea in both eyes (Fig. 2A); the remainder of the retina was normal. On FA; the spoke-wheel appeared hypointense, the AF level in the posterior pole was otherwise normal (Fig. 2B). The GVF was noted to be normal in either eye at 135°×105° and 120°×90° for III-4e and I-4e stimulus targets, respectively. The ERG results showed normal dim-light scotopic response (DA 0.01) and normal cone responses; the combined maximal responses (DA 2.29 and DA 7.6) showed low-normal a-wave amplitudes (Fig. 3B). The SD-OCT demonstrated reduction in schitic changes on the ELM (* in Fig. 2G). The central subfield thickness was 429 and 478 μm in the right and left eyes, respectively. At the most recent follow-up visit at 21 years, BCVA was noted to be 20/25 and 20/30 in the right and left eyes, respectively. Field evaluation showed normal macular reflex with normal macular reflex with macular reflex. At the most recent follow-up visit at 21 years, BCVA was noted to be 20/25 and 20/30 in the right and left eyes, respectively. Field evaluation showed normal macular reflex with normal macular reflex with macular reflex. At the most recent follow-up visit at 21 years, BCVA was noted to be 20/25 and 20/30 in the right and left eyes, respectively. Field evaluation showed normal macular reflex with normal macular reflex with macular reflex. At the most recent follow-up visit at 21 years, BCVA was noted to be 20/25 and 20/30 in the right and left eyes, respectively. Field evaluation showed normal macular reflex with normal macular reflex with macular reflex. At the most recent follow-up visit at 21 years, BCVA was noted to be 20/25 and 20/30 in the right and left eyes, respectively. Field evaluation showed normal macular reflex with normal macular reflex with macular reflex. At the most recent follow-up visit at 21 years, BCVA was noted to be 20/25 and 20/30 in the right and left eyes, respectively. Field evaluation showed normal macular reflex with normal macular reflex with macular reflex. At the most recent follow-up visit at 21 years, BCVA was noted to be 20/25 and 20/30 in the right and left eyes, respectively. Field evaluation showed normal macular reflex with normal macular reflex with macular reflex. At the most recent follow-up visit at 21 years, BCVA was noted to be 20/25 and 20/30 in the right and left eyes, respectively. Field evaluation showed normal macular reflex with normal macular reflex with macular reflex. At the most recent follow-up visit at 21 years, BCVA was noted to be 20/25 and 20/30 in the right and left eyes, respectively. Field evaluation showed normal macular reflex with normal macular reflex with macular reflex. At the most recent follow-up visit at 21 years, BCVA was noted to be 20/25 and 20/30 in the right and left eyes, respectively. Field evaluation showed normal macular reflex with normal macular reflex with macular reflex. At the most recent follow-up visit at 21 years, BCVA was noted to be 20/25 and 20/30 in the right and left eyes, respectively. Field evaluation showed normal macular reflex with normal macular reflex with macular reflex. At the most recent follow-up visit at 21 years, BCVA was noted to be 20/25 and 20/30 in the right and left eyes, respectively. Field evaluation showed normal macular reflex with normal macular reflex with macular reflex. At the most recent follow-up visit at 21 years, BCVA was noted to be 20/25 and 20/30 in the right and left eyes, respectively. Field evaluation showed normal macular reflex with normal macular reflex with macular reflex.
FAF showed a partial (right) or complete (left) spoke-wheel pattern of hyperintense AF; foveolar hyper-AF was noted in the left eye (Figs. 2I, 2J). Fluorescein angiography revealed no abnormalities in the right eye; presence of macular staining with no leakage was noted in the left eye. The ERG showed normal rod and cone responses. On SD-OCT, the right eye showed schitic changes, more so in the inner nuclear layers (Figs. 2K, 2L). In the left eye, macular atrophy was noted (Fig. 2M); both eyes showed disruptions in the photoreceptor IS and OS, and the ELM (* in Figs. 2K, 2L, 2M). The central retinal thickness was
reduced in most ETDRS rings consistent with macular atrophy (left eye); GCL-IPL thickness was either normal (1- to 3-mm ring) or increased (3- to 6-mm ring; Figs. 4A, 4B).

Genetic Results: Family-A

Among the 132 genes tested by sequencing, two likely disease causing variants were observed in \textit{CRB1} (NM_201253) in both siblings. The changes are denoted as c.367G>T/p.Gly123Cys (novel rare variant) and c.2483G>A/p.Cys948Tyr (previously published).\textsuperscript{6,23–26} No deletion or duplication was detected in \textit{RS1} or any of the 141 eye disease associated genes tested. Because FFR was different from other known \textit{CRB1}-associated phenotypes, WES was performed. The filtering steps used in the WES analysis of II-1 and II-2 are summarized in Table 1. In total, 540 nonsynonymous coding variants were classified as rare variants (see Methods).

One hundred seventy-five of these rare coding variants were shared by the two individuals; among these, only nine genes had at least two shared variants amongst both siblings. None of the rare coding sequence variants shared amongst the sibship was in a homozygous state.

All shared SNVs in the nine genes were assessed (19 in total; \textit{PCDHGB7} had 3 variants) and scored using the six predictive tools (see methods section) and cumulative score was calculated (Table 2). A minimum cut-off of 2/6 was set to prioritize pathogenicity of individual SNV. All genes that had two SNVs meeting the cut-off score were prioritized: \textit{CRB1} and \textit{SKOR1} (Table 2).

Both \textit{CRB1} variants (c.367G>T/p.Gly123Cys and c.2483G>A/p.Cys948Tyr) had the maximum predictive score of six. Amongst the nine genes, only \textit{CRB1} was previously associated with a human disease phenotype following a Mendelian pattern of inheritance (Table 1). Taken together, \textit{CRB1} was considered
a strong candidate gene for FFR. The CRB1 variants were verified by Sanger sequencing and both variants segregated with the disease phenotype in the family; parents were carriers (I-1 carried p.Gly123Cys; I-2 carried p.Cys948Tyr). Both SKOR1 variants, c.1897C>T/p.Arg633Trp and c.2260C>G/p.His754Asp (NM_001258024) were determined to be inherited paternally by Sanger sequencing, and thus excluded. The novel exon 2 CRB1 variant c.367G>T/p.Gly123Cys was not found in any of the control databases (1000 genomes, ExAC, NHLBI EVS, CG, dbSNP) and was well conserved in lower vertebrates up to Xenopus (Fig. 1D). The p.Gly123Cys change was predicted to be probably damaging by Polyphen with the highest score of 1.0, and SIFT categorized the change to be deleterious with the maximum score of 0. The variant c.2483G>A/p.Cys948Tyr in exon 9 of CRB1 has earlier been associated with LCA, EORD and early onset RP.7,8,23–28 It is the most frequent CRB1 disease causing variant, and constitutes 152 of 1010 CRB1 alleles registered in the Leiden Open Variation Database (in the public domain, http://databases.lovd.nl/shared/genes/CRB1). The cysteine at position 948 is well conserved in lower vertebrates such as Zebrafish (Fig. 1D).

The functional consequences of the two CRB1 mutations can be rationalized by considering the folding of other epidermal growth factor–like (EGF-like) structures. Epidermal growth factor–like domains are often present in the extracellular domain of membrane-bound proteins or in proteins known to be secreted. The small (~40 amino acid) domains

**TABLE 1.** Whole-Exome Sequencing Filtering Steps in Family-A

<table>
<thead>
<tr>
<th>Filtering Steps</th>
<th>Case II-1</th>
<th>Case II-2</th>
<th>Total Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total variants</td>
<td>91,500</td>
<td>91,935</td>
<td>111,418</td>
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<tr>
<td>Coding variants</td>
<td>19,946</td>
<td>19,758</td>
<td>24,090</td>
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<td>Nonsynonymous coding variants</td>
<td>9,432</td>
<td>9,390</td>
<td>18,822</td>
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<tr>
<td>Coding variants with allele frequency ≤ 0.01 (rare variants)</td>
<td>337</td>
<td>358</td>
<td>695</td>
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<tr>
<td>Shared rare variants (all heterozygous and homozygous)</td>
<td>NA</td>
<td>NA</td>
<td>175</td>
</tr>
<tr>
<td>Genes with ≥ 2 shared rare variants</td>
<td>NA</td>
<td>NA</td>
<td>9</td>
</tr>
<tr>
<td>Genes with 2 shared heterozygous variants</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Chr. 1) CRB1, IGFN1
(Chr.5) PCDHGAS, PCDHGB7
(Chr.15) SKOR1
(Chr. 16) TBL3
(Chr. 17) MYH13
(Chr.19) ZNF780B, ZNF780A

Genes with shared homozygous variants
Genes with known phenotypes

Na, Not applicable; Chr, chromosome.

**FIGURE 4.** Segmentation analysis derived from macular cube scans of SD-OCT. Only left eye data was analyzed. The central 1-mm ring is not shown as it had significant schitic changes that impaired analysis. *Pink circle* and *triangle* represent first and last visits, respectively, in the affected sibling of the proband in Family-A (II-2). *Blue triangle* represents single test visit of the proband in Family-B. Inner corresponds to 1- to 3-mm ring, outer corresponds to 3- to 6-mm ring and macular corresponds to all three rings. (A) Represents total retinal thickness with the exclusion of retinal nerve fiber layer. Macular atrophy is noted in Family-B proband. There is increased retinal thickness in II-2 of Family-A. (B) Thickness of the GC/LPL complex. The layer was consistently thicker in II-2 of Family-A.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome/Genomic Position</th>
<th>RefSeq ID/Exon Number/Nucleotide Substitution/Amino Acid Substitution</th>
<th>Zygosity</th>
<th>Sift Score</th>
<th>Polyphen Score</th>
<th>CADD Phred Score</th>
<th>PhylopP Value</th>
<th>PhyloP Mam Avg</th>
<th>PhyloP Vert Avg</th>
<th>Cumulative Predicted Score (-/6)</th>
<th>ExAC Frequency</th>
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<tbody>
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<td>CRB1</td>
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<td>NM_201253:exon2:c.G367T:p.G123C</td>
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<td>1</td>
<td>15.58</td>
<td>2.854</td>
<td>0.806</td>
<td>1.000</td>
<td>-</td>
<td>0.0002086</td>
</tr>
<tr>
<td>IGFN1</td>
<td>chr1:197403836:G:A</td>
<td>NM_201253:exon9:c.G2843A:p.C948Y</td>
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<td>0.996</td>
<td>15.23</td>
<td>2.479</td>
<td>0.170</td>
<td>0.000000</td>
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<td>Het</td>
<td>0.056</td>
<td>0.922</td>
<td>12.80</td>
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<td>0.160</td>
<td>12.33</td>
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<td>-</td>
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<td>0.260</td>
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<td>0.020</td>
<td>12.33</td>
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<td>0.050</td>
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<td>TBL3</td>
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<td>0.056</td>
<td>0.953</td>
<td>15.58</td>
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<td>0.056</td>
<td>0.780</td>
<td>20.80</td>
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<td>Het</td>
<td>0.056</td>
<td>0.340</td>
<td>15.58</td>
<td>2.854</td>
<td>0.806</td>
<td>1.000</td>
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<td>ZNF780B</td>
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<td>NM_001005851:exon5:c.C2069T:p.T690I</td>
<td>Het</td>
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<td>1.000</td>
<td>15.58</td>
<td>2.854</td>
<td>0.806</td>
<td>1.000</td>
<td>-</td>
<td>0.00000002086</td>
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</table>

Het, heterozygous. Cut-off values to predict pathogenicity for each of the six tools are as follows: Polyphen score ≥ 0.95; SIFT score > 0.05, mutation assessor (Ma) score ≥ 2.0, CADD Phred score ≥ 15, Average PhyloP nucleotide conservation inferred from placental mammal-value ≥ 1.0 (PhyloP Mam Avg), and Average PhyloP nucleotide conservation inferred from vertebrate-value ≥ 1.0 (PhyloP Vert Avg). Bolded cells represent SNV scores that met the pathogenicity cut-off value for a specific predictive tool. Cumulative predicted pathogenicity scores for each SNV were determined and shown in the far right column (-/6).
occur in tandem repeats, forming larger solenoid-like structures that bind calcium.\textsuperscript{29,30} The disulphide bridges are key to the architecture of the EGF-like domains, and add rigidity to each repeat. The extracellular domain of CRB1 contains EGF-like domains, which have a typical structure containing six cysteine residues that form disulfide bridges with each other.\textsuperscript{28} Loss of one of the cysteine residues at position 948 (p.Cys948Tyr) would disrupt formation of EGF-like domain #14 of CRB1 by eliminating a disulphide bond between Cys948 and Cys933 (disulphide denoted by # in Fig. 1C). The other mutation (p.Gly1235Cys) occurs at the base of a turn within EGF-like repeat #5 of CRB1. Owing to steric flexibility, glycine is important to turn structures, and hence the mutant cysteine at position 123 would significantly affect the activity of the EGF-like region to properly form the solenoid-like structure (mutation denoted by * in Fig. 1C).\textsuperscript{31} Because the oligomeric association of the EGF-like domains into the solenoid structures is important for protein function, any disruption of domain formation would affect the cellular role and activity of CRB1. Moreover, the cysteine residue at position 123 may form aberrant disulphide bonds with other cysteine residues, potentially leading to misfolding of the protein.

The WES results of the siblings were assessed for any rare coding sequence variants that could modify the disease phenotype. The CRB complex members (CRB2, CRB3, MUPP1, MPP3, MPP4, MPP5, and PATJ) and any other proteins that form the CRUMBS network (CASK, DENB31, DLG1, DLG4, EPB41L5, INADL, LIN7C, MPDZ, and SDCBP)\textsuperscript{32,33} were analyzed; no variants were shared among the siblings or found in either one of them in heterozygous or homozygous state. The WES results were also assessed for any rare, nonsynonymous, coding sequence variants in any of the known retinal dystrophy genes; no shared variants were observed, single heterozygous variants were seen in II-1 (USH2A and CRX) or in II-2 (CC2D2A; Supplementary Table S2).

**Genetic Results: Family-B**

In II-2, two presumed disease causing variants were observed in CRB1 on WES analysis (c.498_506 del/p.Ile167_Gly169del and c.2290C > T/p.Arg764Cys). Both variants were confirmed by Sanger sequencing and segregated with the disease phenotype (Fig. 1B). The exon 2 variant, c.498_506 del/p.Ile167_Gly169del was not found in any of the control databases and has been earlier associated with early onset RP.\textsuperscript{34} The exon 7 variant, c.2290C > T/p.Arg764Cys has been previously associated with LCA, EORD, and RP.\textsuperscript{4,7,8,23,28} This variant is reported at extremely low frequency in ExAC (0.000099), never in a homozygous state. The residues p.Ile167 and p.Gly169 are conserved until vertebrates such as chicken and elephant respectively; the residues p.Asp168, p.Ile167 and p.Gly169 are conserved until vertebrates such as chicken and elephant respectively; the residues p.Asp168 and p.Ile167 are conserved in Rhesus (Fig. 1D). The deletion of the residues including p.Asp168 would affect the beta-turn of the EGF-like domain (\(^*\) in Fig. 1C). The p.Arg764Cys is located on the second laminin AG-like domain and is poorly conserved. Single heterozygous, rare, coding sequence non-synonymous variants were noted in in BBS7 and CNGB3 (Supplementary Table S2). No rare nonsynonymous coding sequence variants were noted in any of the CRB complex members or in any protein that form part of the CRUMBS network.

**DISCUSSION**

This is the first report implicating CRB1 mutations to underlie FFR and confirms the autosomal recessive inheritance pattern of the disorder.\textsuperscript{1} This report expands the phenotypic spectrum of CRB1-related disorders. The structural improvement (SD-OCT) of foveal schisis following topical Dorzolamide therapy was not associated with any significant improvement of visual function. The disease is of early onset and demonstrates stable vision parameters into early adulthood\textsuperscript{1,2} but may show some deterioration and macular atrophy in later stages.

Schitic/cystoid change at the macula is the diagnostic retinal anomaly in FFR, and found in Family-A, and the right eye of the Family-B proband in the current study. In 2014, Tsang et al.\textsuperscript{9} used WES to establish CRB1 as the genetic basis of an unusual maculopathy in a sibship (1 male, 1 female; p.Arg1331Cys/p.Pro1381Leu) who had mottled granularly-speckled maculopathy along with paramacular annular RPE atrophy extending nasal to the disc. The unusual maculopathy phenotype spared the peripheral retina and one of the individuals also had cystoid macular changes.\textsuperscript{3} Recently, Wolfson et al.\textsuperscript{10} reported female twins with mild foveal RPE mottling, cystoid macular edema and moderately reduced amplitudes of cone full-field ERGs to harbor homozygous p.Pro836Thr mutations in CRB1. CRB1-related retinal dystrophy (CRB1-related RP, EORD, and complex disease (nanophthalmos-RP-optic disc drusen) have also been variably associated with cystic/schitic changes at the macula.\textsuperscript{8,11,26} Taken together, cystoid/schitic macular changes appear to be a feature of CRB1-related retinopathies with the exception of LCA.

Full-field rod and cone ERGs were normal in all affected cases in the current study excluding any generalized rod and cone dysfunction or loss of function, consistent with FFR.\textsuperscript{1} In the maculopathy phenotype described by Tsang et al.,\textsuperscript{9} rod and cone ERG implicit times were delayed which suggested mild generalized cone dysfunction. In the CRB1-related maculopathy phenotype reported by Wolfson et al.,\textsuperscript{10} the ERGs showed a moderate generalized cone dystrophy phenotype. These findings suggest that CRB1-related maculopathies could show a range of electrophysiological phenotypes.

In flies, the Crumbs protein is required for appropriate photoreceptor morphogenesis, assembly of adherens junction, and maintenance of apico-basal photoreceptor cell polarity.\textsuperscript{6,35,36} Mouse mutants models of Crb1 show disruptions in the outer limiting membrane and focal loss of adherens junction; subsequently, focal loss of adhesion between photoreceptors and Müller cells ensue, which leads to displacement of photoreceptors that form pseudorosettes and progressive retinal disorganization.\textsuperscript{37–40} A recent study reports a spontaneous rat mutant of Crb1 to demonstrate extensive cystoid changes in the inner and outer retinal layers in addition to progressive retinal degeneration and retinal telangiectasia.\textsuperscript{41} Increased retinal thickness with loss of lamination is a relatively constant feature of CRB1-related human LCA, EORD, and RP.\textsuperscript{4,7,8,23,28} However, in the current study, retinal lamination appeared normal; retinal laminations was also reported normal in the sibship in the unusual maculopathy phenotype.\textsuperscript{9} The increased central retinal thickness in the sibship (Family-A) in the current study is predominantly a consequence of the persistent schitic changes at the level of inner and outer nuclear layers and GCL. The SD-OCT segmentation analysis showed GCL-IPL complex thickness to be normal (II-1; Family-A, borderline increased (II-2; Family-B), or markedly increased (case II-2; Family-A).

Both p.Cys948Tyr and p.Arg764Cys variants found in this report have been previously associated with LCA, EORD, and RP in both homozygous and compound heterozygous states.\textsuperscript{4,7,8,23–28} The p.Ile167_Gly169del variant has been associated with early onset RP in homozygous and compound heterozygous forms. The p.Pro836Thr variant recently associated with CRB1-related maculopathy (Wolfson et al.\textsuperscript{10}) in homozygous state, has previously been associated with RP (homozygous).\textsuperscript{26}
and EORD (compound heterozygous).\textsuperscript{9} The p.Pro1381Leu variant associated with unusual CRB1-maculaopathy in compound heterozygous state (Tsang et al.)\textsuperscript{9} has previously been associated with LCA (compound heterozygous).\textsuperscript{9} This suggests genetic and/or environmental factors modifying the expression of the CRB1 phenotype. In the present study, no nonconsy-nomous rare coding sequence variants were found in any other CRB proteins or any protein in the CRUMBS network in any of the affected individuals.

Disease-causing mutations of residues present in the EGF-like domains of CRB1 have been described previously. In particular, of the 43 mutations in CRB1 that cause RP (RP12), 17 are present in EGF-like domains, 22 are present in laminin G-like domains and four are outside of these structural regions. Similarly, for LCA (LCA8), 17 mutations occur in the EGF-like domains, 17 in the laminin G-like domains, and 2 are outside of these regions (in the public domain, www.uniprot.org). The severity of each of these mutations would depend highly on the characterization of the mutation (which amino acid substitution occurs, deletion, or premature stop codon) and the role of that particular EGF-like or laminin G-like region in protein-protein interactions or in the formation of multiprotein tandem repeats. How these mutations alter the protein structure and protein interactions likely is what defines the severity and character of the clinical phenotype.

Macular schitic/cystoid changes are also found in a wide variety inherited retinal disorders that include RP, X-linked retinoschisis, enhanced S-cone syndrome, choroideremia, gyrate atrophy, autosomal recessive bestrophinopathy, autosomal recessive maculopathy, and dominant cystoid macular dystrophy.\textsuperscript{1,9,10,42–49} In the majority of the disorders, carbonic anhydrase inhibitors (CAI) have been shown to have modest improvement with CAI.\textsuperscript{9,10} In the present study reports for the first time that CAI reduces schitic changes in FFR. It is noted that other CRB1 maculopaties also show improvement with CAI.\textsuperscript{9,10}

Recently, a new entity termed stellate nonhereditary idiopathic foveal maculoschisis (SNIFR) has been proposed to accommodate sporadic cases of unilateral or bilateral macular schisis; \textbf{94\% (16/17) of the cases were females.}\textsuperscript{51} It is possible that some of these cases represent isolated foveal retinoschisis and may harbor mutations in \textit{CRB1}.

To conclude, FFR is an autosomal recessive condition due to mutations in \textit{CRB1}. This is the first report of a male subject with isolated/familial foveal retinoschisis. Mutations in \textit{CRB1} result in a range of autosomal recessive retinal dystrophies that vary in severity, age of onset, and extent of retinal involvement; FFR represents the mildest end of the spectrum of \textit{CRB1}-related diseases known to date. \textit{CRB1}-related maculopathy phenotypes may or may not show generalized retinal involvement; the ERG helps in its ascertainment. Schitic or cystoid changes may be a frequent finding in \textit{CRB1}-related maculopathy phenotypes. It is intriguing that \textit{CRB1}, a gene cardinal in retinal development causes FFR without generalized retinal dysfunction, the reason for which remains to be elucidated.

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