



HAL
open science

Phenotypic variability in patients with retinal dystrophies due to mutations in CRB1

Robert H H Henderson, Donna S Mackay, Zheng Li, Phillip Moradi, Panagiotis Sergouniotis, Isabelle Russell-Eggitt, Dorothy A Thompson, Anthony G Robson, Graham E Holder, Andrew R Webster, et al.

► **To cite this version:**

Robert H H Henderson, Donna S Mackay, Zheng Li, Phillip Moradi, Panagiotis Sergouniotis, et al.. Phenotypic variability in patients with retinal dystrophies due to mutations in CRB1. *British Journal of Ophthalmology*, 2010, 95 (6), pp.811. 10.1136/bjo.2010.186882 . hal-00595938

HAL Id: hal-00595938

<https://hal.science/hal-00595938v1>

Submitted on 26 May 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 TITLE: Phenotypic variability in patients with retinal dystrophies due to mutations in *CRB1*

2

3 AUTHORS:

4 1. Robert H Henderson 1,2,3 MRCOphth MD (Corresponding author)

5 2. Donna S Mackay 2 PhD

6 3. Zheng Li 2 PhD

7 4. Phillip Moradi 1,2,3 MRCOphth

8 5. Panagiotis Sergouniotis 1,2

9 6. Isabelle Russell-Eggitt 4 FRCOphth

10 7. Dorothy A Thompson 4 PhD

11 8. Anthony G Robson 1,2 PhD

12 9. Graham E Holder 1,2 PhD

13 10. Andrew R Webster 1,2 MD FRCOphth

14 11. Anthony T Moore 1,2,3 FRCOphth FMedSci

15

16 Institutions:

17 1) Moorfields Eye Hospital, 162 City Road, London EC1V 2PD

18 2) UCL Institute of Ophthalmology, 11-36 Bath Street, London. EC1V 9EL

19 3) Great Ormond Street Hospital for Children, Great Ormond Street, London. WC1N

20 3JH

21 4) Ulverscroft vision research group, Great Ormond Street Hospital for Children, Great

22 Ormond Street, London. WC1N 3JH

23 Corresponding author address: Professorial Unit, Moorfields Eye Hospital, 162 City Road,

24 London. EC1V 2PD r.henderson@ucl.ac.uk tel: +44 207 566 2260 fax: +44 207 608 6830

25

26

27 **ABSTRACT:**

28 **Objectives:** To identify CRB1 mutations in a large cohort of patients with recessive retinal
29 dystrophies and to document the retinal phenotype and visual prognosis.

30

31 **Design:** A hospital based cross sectional study of children and adults with recessive retinal
32 dystrophies.

33

34 **Participants:** Three hundred and six patients with Leber's congenital amaurosis (LCA),
35 early onset childhood retinal dystrophy (EORD), or juvenile onset retinitis pigmentosa (arRP)
36 were recruited to the study and gave blood samples for molecular genetic analysis

37

38 **Methods:** A detailed clinical examination was performed including: LogMAR visual acuity,
39 refraction, Goldmann visual fields, slit-lamp biomicroscopy, fundus photography,
40 autofluorescence imaging and optical coherence tomography. The results of
41 electrophysiology testing were available in all patients. DNA was obtained for molecular
42 genetic analysis. Initial screening for mutations was performed using the LCA chip (Asper
43 Ophthalmics). Patients who had one or more CRB1 mutations identified on the chip, and
44 other patients whose phenotype suggested a CRB1 genotype, underwent direct sequencing. In
45 addition, consanguineous families segregating recessive RP underwent a whole genome scan
46 using Affymetrix gene chips (Affymetrix UK Ltd) and affected family members showing
47 linkage to the RP12 locus underwent sequencing of the CRB1 gene.

48

49 **Main outcome measures:** Identification of patients with mutations in CRB1 and detailed
50 documentation of the clinical phenotype

51

52 **Results:** Mutations in CRB1, including 17 novel mutations, were identified in 41 patients
53 from 32 families. We identified both disease mutations in 34 patients from 26 families and
54 these patients underwent detailed phenotyping. Common phenotypic features included
55 hypermetropic refractive error; nummular pigmentation at the level of the RPE; and increased
56 retinal thickness on OCT. Most patients had a clinical and electrophysiological phenotype
57 consistent with a diagnosis of LCA or rod-cone dystrophy but three patients had ERG
58 evidence of cone-rod degeneration. A minority of patients developed peripheral retinal
59 telangiectasia, which in some cases led to seclusio pupillae and angle closure glaucoma.

60

61 **Conclusion:** Mutations in CRB1 are associated with a range of recessively inherited retinal
62 dystrophies including LCA, childhood and juvenile onset rod-cone, and cone-rod dystrophies.
63 Although the phenotype is usually severe, in milder cases there is a window of opportunity
64 for therapeutic intervention in early childhood.

65

66 Retinitis Pigmentosa (RP) is a generic term used to describe a group of inherited retinal
67 degenerations that lead to death of rod and cone photoreceptors. RP has a prevalence of about
68 1 in 3-4000. It is clinically and genetically heterogeneous with autosomal dominant,
69 autosomal recessive and X-linked inheritance being described.[1] Even amongst these
70 subtypes there is considerable genetic heterogeneity with over 100 genes currently known
71 (<http://www.sph.uth.tmc.edu/retnet/>). One of the challenges for the clinician is how to utilise
72 the results of molecular genetic testing to improve clinical care and our understanding of
73 disease mechanisms. Detailed knowledge of genotype/phenotype correlations allows targeted
74 and therefore cost-effective molecular testing, clearly advantageous in a disorder with so
75 many causative genes, and will help determine which disorders should be prioritised for the
76 development of novel therapies.

77

78 Retinitis Pigmentosa with preservation of the para-arteriolar RPE(PPRPE) was first described
79 by Heckenlively et al in 1982.[2] In this disorder there is relative preservation of retinal
80 pigment epithelium (RPE) adjacent to and under retinal arterioles. In 1994, van den Born et
81 al[3] reported a clinical study of 22 patients from one large family with PPRPE. Subsequent
82 molecular genetic testing identified the disease locus on 1q31-32.1[4] and den Hollander et
83 al[5] subsequently identified *CRB1* as the causative gene.

84

85 The *CRB1* gene is analogous to the *Drosophila Melanogaster* Crumbs protein that is involved
86 in the establishment of the polarity of epithelial cells.[6,7] The CRB1 protein is found at the
87 apical region of all retinal epithelial cells, the rod and cone photoreceptor cells and Muller
88 glial cells(MGCs) in the adult mouse retina.[8] It co-localises with the zonula-adherens(ZA)
89 and is a major component of the outer limiting membrane. Mutations in *CRB1* have been

90 identified in a variety of human retinal disorders including Leber congenital amaurosis
91 (LCA),[9-11] PPRPE,[10] and retinitis pigmentosa.[12,13]

92

93 The aim of this study was to identify mutations in *CRBI* in a large cohort of patients and to
94 characterise the ocular phenotype.

95

96

97 METHODS

98 **Patient recruitment:** Patients with a diagnosis of LCA or early childhood onset retinal
99 dystrophy(EORD), and families with autosomal recessive RP where there was parental
100 consanguinity were recruited from two centres – Moorfields Eye Hospital and Great Ormond
101 Street Hospital for Children, London, UK. Ethics committee approval was obtained and
102 research procedures were carried out in accordance with institutional guidelines and the
103 declaration of Helsinki. Informed consent was obtained for all subjects prior to their
104 participation in the study.

105

106 **Clinical Definitions:** All subjects had a family history consistent with AR inheritance.
107 Patients were diagnosed as having LCA if vision was poor or absent in the first 3 months
108 after birth, and where there was no recordable electroretinogram. A diagnosis of early onset
109 retinal dystrophy was made when symptoms and/or signs were present before the age of 5
110 years and subsequently, on electroretinography, there was evidence of a severe cone-rod or
111 rod-cone dystrophy. Children older than 5 years with later onset of symptoms and signs of a
112 retinal dystrophy were termed ‘juvenile onset’ or were labelled synonymously as autosomal
113 recessive retinitis pigmentosa(ARRP).

114

115 **Clinical Evaluation:** A full clinical history and examination was performed in all subjects. In
116 older children and adults this included best corrected LogMAR visual acuity and colour
117 vision testing using the Hardy-Rand-Rittler pseudoisochromatic plates(Richmond Products).
118 In the low vision range, for the purposes of later analysis, LogMAR values of 2.0 were
119 applied for counting fingers acuity; 3.0 for hand movements, 5.0 for light perception and 6.0
120 for no light perception.[14] Visual field testing using Goldmann perimetry was performed in

121 those with stable fixation. Subsequent analysis of the visual field area was performed using
122 planimetry within the V4e isopter.

123

124 Refraction data were ascertained either by obtaining an objective cycloplegic refraction using
125 neutralising lenses, or using an autorefractor(Luneau L62-3D autorefractokeratometer -
126 LUNEAU Chartres, France). Fundus photographs were obtained where possible using the
127 Topcon retinal camera(TRC-50IX with IMAGEnet 2000 system software – TOPCON
128 corporation, Japan). Optical Coherence Tomography scans were performed on a Stratus
129 OCT™(Carl Zeiss Meditec, Inc. Dublin, CA); further OCT imaging was performed in some
130 patients using the Topcon 3d OCT-1000 and the Spectralis spectral domain OCT(Heidelberg
131 engineering, Germany). Fundus autofluorescence images were obtained using the Heidelberg
132 Retinal Angiograph-II(Heidelberg engineering, Germany). Axial length and keratometry
133 measurements were obtained using the IOL Master(Carl Zeiss Meditec, Dublin CA). All
134 patients had electrophysiological testing performed either at our institutions or at the referring
135 hospital: many recordings were historical and either preceded ISCEV Standard recordings or
136 incorporated the ISCEV standards pertaining at the time. Where further electrophysiological
137 testing was required to make or confirm a diagnosis, adults and older children had
138 electroretinography conducted in accordance with current ISCEV standards using gold foil or
139 DTL electrodes.[15,16] ERG testing for infants and young children in our institutions was
140 performed using skin electrodes in accordance with previously published protocols.[17,18]

141

142

143

144

145

146 **Genotyping**

147 DNA was extracted from whole blood obtained from each affected individual and immediate
148 family members using the Nucleon BACC-2 kit(GE Healthcare) performed as per
149 manufacturer's instructions.

150

151 DNA samples from patients with LCA and EORD were sent to Asper Ophthalmics(Tartu,
152 Estonia) for analysis using the LCA chip.[19] In those subjects in whom a mutation in *CRBI*
153 was identified, DNA samples were then sequenced to confirm the chip findings and, where
154 only one mutation was found, to identify a second change.

155

156 DNA from consanguineous families with autosomal recessive RP were analysed using the
157 Affymetrix 100K gene chip. Those showing linkage to the 1q31-32 region were sequenced
158 for *CRBI* mutations. DNA samples from patients with recessive RP who had a retinal
159 phenotype characteristic of *CRBI* were also sequenced.

160

161 Oligonucleotide primer pairs (appendix 1) were created to cover the exon and splice site
162 junctions (based on RefSeq accession number NM_201253.1) and were designed using
163 Primer 3(http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and ordered from
164 Sigma-Genosys(Sigma–Aldrich, UK). The amplified products were sequenced with Big Dye
165 terminator v1.1 cycle sequencing kits(Applied Biosystems, Cheshire, UK) according to
166 manufacturer's instructions. The pathogenic impact of the *CRBI* sequence variants caused by
167 non-synonymous changes of the protein sequence was evaluated through SIFT
168 (<http://sift.jcvi.org>) and PolyPhen(<http://genetics.bwh.harvard.edu/pph/index.html>) sequence
169 homology-based programmes.

170

171 RESULTS

172 DNA samples from 250 probands with LCA/EORD were analysed using the LCA chip. 21
 173 probands were found to have mutations in *CRB1*; 7 had two mutations and 14 had one
 174 mutation identified. All mutations were confirmed by direct sequencing and a second
 175 mutation was identified in 11 patients. Two recessive RP families, who were studied with
 176 Affymetrix 100k gene chips, showed linkage to the RP12 locus and were found to have
 177 homozygous mutations in *CRB1*. A further 14 RP patients with a clinical phenotype
 178 suggestive of *CRB1* related disease were also sequenced; 9 were found to have mutations in
 179 *CRB1* and two mutations were identified in 6 of these patients.

<i>Patient No.</i>	<i>Diagnosis</i>	<i>Exon</i>	<i>Nucleotide 1</i>	<i>Protein 1</i>	<i>Exon</i>	<i>Nucleotide 2</i>	<i>Protein 2</i>
1	EORCD	9	2843G>A	C948Y			
2	LCA	2	614T>C	I205T			
3	EORD	9	3037C>T*	Q1013X*			
4	ARRP	7	2129A>T	E710V*			
5	ARRP + retinal telangiectasia	7	2290C>T	R764C			
6,7	ARRP/LCA	9	3103C>T*	H1035Y*			
8,9	EORCD	7	2401A>T	K801X	12	4094C>A*	A1365D*
10-15	EOCRD/EORCD/ARRP + retinal telangiectasia	3	750T>G	C250W	3	750T>G	C250W
16	LCA	7	2548G>A	G850S	9	3307G>C	G1103R
17	LCA	7	2401A>T	K801X	9	2843G>A	C948Y
18	EORCD	7	2290C>T	R764C	7	2401A>T	K801X
19	LCA	9	3542dupG*	C1181WfsX12*	9	3542dupG*	C1181WfsX12*
20	EORCD	9	2843G>A	C948Y	7	2222T>C	M741T
21	LCA	9	2843G>A	C948Y	3	717_718InsG*	Q240fsX20*
22	LCA	7	2548G>A	G850S	12	4142C>T*	P1381L*
23	LCA	9	2843G>A	C948Y	9	3655T>G*	C1174G*
24	LCA	9	2843G>A	C948Y	6	1576C>T*	R526X*

25	LCA	9	2843G>A	C948Y	9	2843G>A	C948Y
26	EORCD	9	2843G>A	C948Y	9	2843G>A	C948Y
27,28	LCA	7	2676 delG*	K892NfsX95*	7	2676delG*	K892NfsX95*
29	LCA	7	2548G>A	G850S	9	3008T>C*	I1003T*
30	ARRP	9	2843G>A	C948Y	7	2129A>T*	E710V*
31	LCA	9	2842G>A	C948Y	6	2043G>A*	C681X*
32	ARRP	7	2129A>T	E710V*	7	2234C>T	T745M
33	ARRP	7	2401A>T	K801X	9	3035T>C*	L1012S*
34	ARRP + retinal telangiectasia	9	3074 G>A*	S1025A*	9	3074 G>A*	S1025A*
35,36	EORCD	7	2536G>A	G846R	7	2536G>A	G846R
37	EORCD	2	470G>C*	C157S*	7	2506C>A	P836T
38	EORCD + retinal telangiectasia	4	936T>G*	N312K*	9	2843G>A	C948Y
39	LCA	7	2401A>T	K801X	9	3320T>C	L1107P
40	ARRP + retinal telangiectasia	6	2025G>T*	W675C*	9	3520 T>G	C1174G
41	EORCD+ retinal telangiectasia	7	2234C>T	T745M	8	2688T>A	C896X

180 Table 1

181

182 Of the cohort of 306 patients, 41 patients from 32 families were identified as having
183 mutations of *CRBI*(Table 1). Thirty different sequence variants were identified, two thirds of
184 which were in exons 7 & 9. Seventeen novel disease associated sequence variants were
185 found. None of these variants were found in 100 normal controls, and appropriate segregation
186 of alleles in family members was confirmed where DNA was available. The most common
187 mutation identified was the p.C948Y variant which was present in 13 alleles of 11 patients.
188 Six probands had one mutation found and in 26 probands both alleles were identified. Of the
189 latter group, 7 had homozygous mutations and 19 had compound heterozygous mutations.
190 Mutations in the *CRBI* gene were identified in 7% of the LCA/EORD cohort.

191

192 **Clinical Findings**

193 Thirty-four patients from 26 families had two CRB1 mutations identified. They ranged in age
194 from 2-48 years(mean 19 years; 22 male, 12 female). Twenty-one patients were Caucasian,
195 11 were from the Indian subcontinent and 2 were African. The median visual acuity for the
196 whole cohort was 1.6 LogMAR. The median and range of LogMAR visual acuity
197 measurements for each decade, are given in Table 2.

198

Age range (years)	0-10 (n=7)	11-20 (n=15)	21-30 (n=5)	31-40 (n=4)	41-50 (n=3)
Median LogMAR VA	1	0.95	2	3.5	5.5
LogMAR VA range	0.21 - 5(LP)	0.25 - 5 (LP)	0.8-3(HM)	0.34 - 6 (NLP)	5 - 6 (LP- NLP)

199 Table 2

200

201 The median spherical equivalent refraction for all patient measured was +1.5 dioptries.
202 Funduscopy revealed the majority of patients to have nummular pigmentation at the level of
203 the retinal pigment epithelium that was more widespread in the older patients. Ten
204 patients(29%) had macular atrophy and 6(18%) had PPRPE. Five(15%) subjects had
205 peripheral retinal telangiectasia, which in three cases was associated with exudative retinal
206 detachment. Two patients had seclusio pupillae and secondary glaucoma. Median foveal
207 thickness on Stratus OCT was 326µm(range 212-854µm; s.d. 148µm).

208

209

210

Diagnosis	Median age at examination (years)	LogMAR acuity	Spherical equivalent refraction Dioptres	Visual field size (square degrees inside V4e isopter)	Axial length mm	Foveal thickness on Stratus OCT (μm)	PPRPE	Macular atrophy	'Coats-like' vasculopathy
LCA (n=15)	10	2 (CF)	+6	100	18.70	264	N=2	N=7	N=1
RCD (n=7)	17	2 (CF)	+2	2500	20.2	316	N=1	N=3	N=1
CRD (n=3)	16	0.6	0	-	20.6	235	N=1	N=0	N=1
ARRP (n=9)	20	0.8	+1	4000	20.7	345	N=2	N=0	N=2

211 Table 3

212

213 The LCA patients all had a non-detectable ERG. Visual acuity ranged from 0.9 LogMAR to
214 NPL. Refraction ranged from +4.5 to +9.5 dioptres (spherical equivalent), and correlated with
215 short axial length, which ranged from 17.95mm to 23.06mm(Table 3). Colour vision was
216 absent in all but one patient who was able to discern the highest contrast HRR plates.
217 Reliable OCT measurements could only be made in 6 patients because of nystagmus; foveal
218 thickness ranged from 212 μm to 854 μm with intraretinal cystic spaces seen in two patients.
219 Funduscopy showed white dots at the level of the RPE in the younger patients and deep
220 nummular pigment in older subjects(Figure 1D). Macular atrophy was present in 7 of the
221 15(46%) patients with LCA(Figure 1F). Analysis of mutations in this group of patients
222 showed that 23/24 alleles were either protein truncating(9/24) - both nonsense and frame-
223 shifting - or predicted by both SIFT and PolyPhen analysis to severely affect protein function
224 (14/24).

225

226 The early onset rod-cone dystrophy patients were between 5 and 39 years of age when
227 examined. Nine patients had a historical diagnosis of ARRP, but on further questioning had
228 symptoms that were childhood or juvenile in onset. The median onset of symptoms was at 2.5
229 years(range 6 months to 5 years). The median age at diagnosis was 6 years. Visual acuities
230 ranged from 0.16 LogMAR to PL. The spherical equivalent refractive error ranged from
231 -0.5D to +6.5D with the majority having low hyperopia. Axial lengths ranged from 19.03mm
232 to 23.35mm. The foveal thickness measurements, on Stratus OCT, ranged from 217-396µm.
233 Intraretinal cysts were common. (Figure2B,C,D). The visual field was relatively well
234 preserved in several patients and field area ranged from 102-10000 square degrees. Fundus
235 examination revealed widespread pigment clumping at the level of the RPE in the majority of
236 cases. One patient exhibited PPRPE (Figure1C).

237

238 The 3 patients with cone-rod dystrophy were aged 13, 17 and 19 years at examination. Poor
239 central vision was the first symptom with later development of night blindness and visual
240 field loss. One patient had peripheral retinal telangiectasia and exudative retinal detachment
241 (Figure1A; Figure2E). The ERG findings suggested generalised photoreceptor dysfunction
242 with evidence of more severe macular involvement (Figure3).

243

244 Examination of the mutations in these two groups of early onset patients showed 16/18 alleles
245 would be predicted to affect protein function severely, similar to the pattern seen in the LCA
246 group. Three of 18 alleles contained nonsense mutations; 13/18 alleles contained mis-sense
247 mutations predicted to have a severe effect on protein function.

248

249 The patients with juvenile onset rod-cone dystrophy had a less severe phenotype and later
250 onset of disease. The median age at diagnosis was 10 years. Visual acuities ranged from 0.28

251 LogMAR to NPL, with the median acuity 0.8 LogMAR. Low hypermetropia was noted in all
252 but one patient. Visual field planimetry ranged in size from 620-11160 square degrees.
253 Foveal thickness, measured by OCT, ranged from 322-544 μm with several patients
254 exhibiting intra-retinal cysts. The retinal appearances were more heterogeneous and ranged
255 from a grey discolouration of the RPE around the arcades with PPRPE to extensive retinal
256 pigmentation. Retinal telangiectasia was seen in 2 patients who subsequently developed
257 retinal detachment, uveitis with seclusio pupillae, and angle closure glaucoma. In this group
258 of patients there was a lower prevalence of protein truncating mutations and a higher
259 prevalence of missense mutations compared to those subjects with LCA.

260

261 DISCUSSION

262

263 The present study investigated the incidence of *CRBI* mutations in a large cohort of patients
264 with recessive retinal dystrophies reports 17 novel mutations. An APEX microarray was used
265 as an initial screening strategy and *CRBI* mutations were identified in 7% of patients with
266 LCA and EORD. This is likely to be an underestimate, as sequencing of *CRBI* was not
267 performed in all cases. When cases with a suggestive clinical phenotype were also included
268 the detection rate in this cohort increased to 12%, indicating that *CRBI* mutations are a
269 common cause of LCA and childhood onset rod cone dystrophies.

270

271 Mutations in *CRBI* account for 10-13% of patients with LCA,[9-11] but *CRBI* mutations
272 have also been identified in patients with autosomal recessive retinitis pigmentosa
273 (RP12)[5,20] where there is often para-arteriolar sparing of the RPE. A *CRBI* mutation has
274 also been reported in a single family with autosomal dominant pigmented paravenous
275 chorioretinal atrophy (PPCRA) that exhibited variable expressivity.[21] Peripheral retinal

276 telangiectasia and associated exudation is more commonly associated with *CRBI* mutations
277 than other forms of RP.[3,13] We observed telangiectasis, exudation and subsequent
278 development of retinal detachment in 15% of our cohort. It seems plausible that this ‘Coats-
279 like’ exudative vasculopathy is secondary to a breakdown in the normal blood retinal barrier
280 mediated by loss of normal zonula occludens function and therefore immune exposure to
281 retinal antigen.[22] The characteristic appearances of exudation and retinal detachment,
282 yellow lipid deposition, with subsequent uveitis and neovascular glaucoma would seem to be
283 a final common pathway of abnormal retinal development whether the cause is secondary to
284 mutations in *CRBI*, *Norrin*, or associated with FEVR and Coats disease.

285

286 We identified *CRBI* mutations in patients with LCA, early onset rod-cone dystrophy, juvenile
287 onset RP, and cone rod dystrophy. The severity of the retinal disease generally correlated
288 with the age of onset of symptoms. The disease appears clinically progressive though
289 longitudinal data was not collected; however, all patients examined who were in their fourth
290 decade had visual acuities of CF or worse. There is a readily recognisable phenotype that can
291 help guide molecular genetic testing. In infants there are typically widespread sub-retinal
292 white dots and macular atrophy is common. The characteristic deep nummular pigmentation
293 usually develops in later childhood and the pigmentation becomes more extensive with age.
294 PPRPE, present in 18% of our patients, is strongly suggestive of *CRBI* related disease,
295 particularly when accompanied by peripheral telangiectasia. A variety of macular changes
296 were found: macular atrophy and loss of central macular volume on OCT was noted
297 particularly in patients with LCA and early onset rod-cone dystrophy. No clear relationship
298 with genotype was established though it was noted that 6 of the 13 patients in the cohort that
299 had either heterozygous or homozygous frame-shifting/termination mutations had macular
300 atrophy noted on funduscopy.

301

302 OCT imaging is also extremely helpful in identifying *CRB1* related disease. Jacobson et
303 al[23] first described increased retinal thickness and loss of the normal retinal laminae in
304 patients with *CRB1* mutations. All of the patients in the current study who had OCT showed
305 significantly increased retinal thickness. Newer spectral domain OCT imaging permits better
306 visualisation of the laminar arrangement of the inner retina. In one family, with three affected
307 individuals, spectral domain OCT showed well delineated retinal layers with preservation of
308 the outer limiting membrane (OLM) in the younger patients but coarse lamination and loss of
309 the OLM in the oldest subject (Figure2B,C,D). This suggests that the coarse lamination seen
310 on OCT may not represent a developmental abnormality, but that loss of the normal
311 architecture develops with time. The *CRB1* protein is known to play a critical role in retinal
312 development[6,8,24] and patients with *CRB1* mutations have abnormal retinal structure as a
313 consequence. Longitudinal studies are required to determine whether there is progressive loss
314 of the OLM, and what role this may have in determining the increased retinal volume seen on
315 OCT.

316

317 The preliminary results of gene replacement therapy for LCA associated with mutations in
318 *RPE65* have been promising[27-29] and this has led to optimism that similar treatment
319 strategies may be helpful in other forms of LCA and childhood retinal dystrophy. The retinal
320 disease associated with *CRB1* mutations is generally more severe but there are some patients
321 in whom there is a window of opportunity for therapy in early childhood.

322

323

324 The Corresponding Author has the right to grant on behalf of all authors and does grant on
325 behalf of all authors, an exclusive licence (or non exclusive for government employees) on a
326 worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if
327 accepted) to be published in BJO editions and any other BMJ PGL products to exploit all

328 subsidiary rights, as set out in our licence:
329 (<http://group.bmj.com/products/journals/instructions-for-authors/licence-forms/>)
330

331

- 332 1. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet* 2006;368(9549):1795-
333 1809.
- 334 2. Heckenlively JR. Preserved para-arteriole retinal pigment epithelium (PPRPE) in retinitis
335 pigmentosa. *Br.J.Ophthalmol.* 1982;66(1):26-30.
- 336 3. van den Born LI, van Soest S, van Schooneveld MJ, et al. Autosomal recessive retinitis
337 pigmentosa with preserved para-arteriolar retinal pigment epithelium.
338 *Am.J.Ophthalmol.* 1994;118(4):430-439.
- 339 4. van Soest S, van den Born LI, Gal A, et al. Assignment of a gene for autosomal recessive
340 retinitis pigmentosa (RP12) to chromosome 1q31-q32.1 in an inbred and genetically
341 heterogeneous disease population. *Genomics* 1994;22(3):499-504.
- 342 5. den Hollander AI, ten Brink JB, de Kok YJ, et al. Mutations in a human homologue of
343 *Drosophila crumbs* cause retinitis pigmentosa (RP12). *Nat.Genet.* 1999;23(2):217-
344 221.
- 345 6. Tepass U. Adherens junctions: new insight into assembly, modulation and function.
346 *Bioessays* 2002;24(8):690-695.
- 347 7. Tepass U, Tanentzapf G, Ward R, et al. Epithelial cell polarity and cell junctions in
348 *Drosophila*. *Annu.Rev.Genet.* 2001;35:747-784.
- 349 8. Pellikka M, Tanentzapf G, Pinto M, et al. Crumbs, the *Drosophila* homologue of human
350 CRB1/RP12, is essential for photoreceptor morphogenesis. *Nature*
351 2002;416(6877):143-149.
- 352 9. Lotery AJ, Jacobson SG, Fishman GA, et al. Mutations in the CRB1 gene cause Leber
353 congenital amaurosis. *Arch.Ophthalmol.* 2001;119(3):415-420.
- 354 10. den Hollander AI, Davis J, van der Velde-Visser SD, et al. CRB1 mutation spectrum in
355 inherited retinal dystrophies. *Hum.Mutat.* 2004;24(5):355-369.
- 356 11. Hanein S, Perrault I, Gerber S, et al. Leber congenital amaurosis: comprehensive survey
357 of the genetic heterogeneity, refinement of the clinical definition, and genotype-
358 phenotype correlations as a strategy for molecular diagnosis. *Hum.Mutat.*
359 2004;23(4):306-317.
- 360 12. Khan JA, Ide CH, Strickland MP. Coats'-type retinitis pigmentosa. *Surv.Ophthalmol.*
361 1988;32(5):317-332.
- 362 13. den Hollander AI, Heckenlively JR, van den Born LI, et al. Leber congenital amaurosis
363 and retinitis pigmentosa with Coats-like exudative vasculopathy are associated with
364 mutations in the crumbs homologue 1 (CRB1) gene. *Am.J.Hum.Genet.*
365 2001;69(1):198-203.
- 366 14. Holladay JT. Visual acuity measurements. *J.Cataract Refract.Surg.* 2004;30(2):287-290.
- 367 15. Holder GE, Brigell MG, Hawlina M, et al. ISCEV standard for clinical pattern
368 electroretinography--2007 update. *Doc.Ophthalmol* 2007;114(3):111-116.
- 369 16. Marmor MF, Fulton AB, Holder GE, et al. ISCEV Standard for full-field clinical
370 electroretinography (2008 update). *Doc.Ophthalmol* 2009;118(1):69-77.
- 371 17. Holder GE, Robson AG. Paediatric Electrophysiology: A Practical Approach. In: Lorenz
372 B, Moore AT, editors. *Paediatric Ophthalmology, Neuro-ophthalmology, Genetics.*
373 Heidelberg: Springer, 2006:133-155.
- 374 18. Kriss A, Thompson D. Visual Electrophysiology. In: Taylor D, editor. *Paediatric*
375 *ophthalmology.* Oxford: Blackwell, 1997:93-121.
- 376 19. Henderson RH, Waseem N, Searle R, et al. An assessment of the apex microarray
377 technology in genotyping patients with leber congenital amaurosis and early-onset
378 severe retinal dystrophy. *Invest Ophthalmol.Vis.Sci.* 2007;48(12):5684-5689.
- 379 20. Bernal S, Calaf M, Garcia-Hoyos M, et al. Study of the involvement of the RGR, CRPB1,
380 and CRB1 genes in the pathogenesis of autosomal recessive retinitis pigmentosa.
381 *J.Med.Genet.* 2003;40(7):e89.

382 21. McKay GJ, Clarke S, Davis JA, et al. Pigmented paravenous chorioretinal atrophy is
383 associated with a mutation within the crumbs homolog 1 (CRB1) gene. Invest
384 Ophthalmol Vis.Sci. 2005;46(1):322-328.

385 22. Solomon A, Banin E, Anteby I, et al. Retinitis pigmentosa, Coats disease and uveitis. Eur
386 J Ophthalmol 1999;9(3):202-205.

387 23. Jacobson SG, Cideciyan AV, Aleman TS, et al. Crumbs homolog 1 (CRB1) mutations
388 result in a thick human retina with abnormal lamination. Hum.Mol.Genet.
389 2003;12(9):1073-1078.

390 24. Tepass U. Crumbs, a component of the apical membrane, is required for zonula adherens
391 formation in primary epithelia of Drosophila. Dev.Biol. 1996;177(1):217-225.

392 25. Vallespin E, Cantalapiedra D, Riveiro-Alvarez R, et al. Mutation screening of 299
393 Spanish families with retinal dystrophies by Leber congenital amaurosis genotyping
394 microarray. Invest Ophthalmol Vis.Sci. 2007;48(12):5653-5661.

395 26. Yzer S, Leroy BP, De Baere E, et al. Microarray-based mutation detection and
396 phenotypic characterization of patients with Leber congenital amaurosis. Invest
397 Ophthalmol.Vis.Sci. 2006;47(3):1167-1176.

398 27. Hauswirth WW, Aleman TS, Kaushal S, et al. Treatment of leber congenital amaurosis
399 due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene
400 vector: short-term results of a phase I trial. Hum Gene Ther 2008;19(10):979-990.

401 28. Bainbridge JW, Smith AJ, Barker SS, et al. Effect of gene therapy on visual function in
402 Leber's congenital amaurosis. N.Engl.J.Med. 2008;358(21):2231-2239.

403 29. Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for
404 Leber's congenital amaurosis. N.Engl.J.Med. 2008;358(21):2240-2248.

405
406

Acknowledgments:

407 Fight for Sight, British Retinitis Pigmentosa Society, Special Trustees of Moorfields Eye
408 Hospital, Ulvercroft Foundation, Foundation Fighting Blindness USA, and NIHR
409 (Moorfields Eye Hospital Biomedical Research Centre)

410

411 **Financial Disclosure:**

412 None of the authors have any financial interests to disclose.

413 **Competing Interest:** None declared.

414
415
416
417
418
419
420
421

EXON/ AMPLIME	5' FORWARD	3' REVERSE	ANNEALIN G TEMP
------------------	------------	------------	--------------------

R			
1	CCTCCCGTGTAAGTGATGCT	AACTGACTGTTCACATTGACTG G	60.6
2.1	GCAGCACAAAGGTCACAAAG	GCAGGGATCTGAAGCACATT	65.8
2.2	GGAGGTATTTGCCATCAGGA	TGACAGCAGGCAACCACTTA	62.5
3	CTCTGCCTTGTGCAACTTC	TAAGCCGAGAACGTGAGAGC	64.3
4	CCATGGGTCTTGGGTTGATA	GCAGTAAATATGTGTGAGCAC TGA	64.3
5	CCTCCTTTTAGGCAAATGCTC	TACTTTTCATGCCACAAGG	58.7
6.1	GCTATTCATGCACTTCTGCAA	GCTCCAGCTTCACAAACACA	60.6
6.2	ACTTTTCATTTGAGGGCGATG	ACAAGGTTGGCTTTCACACC	64.3
6.3	GATGGAGAGTGGCATTTCGT	TTTCATAGCAGGCAGAAGCA	64.3
7.1	TTCGTCTTCCATCCCTTCTG	GTCAGGTAGGCCACCAATGT	64.3
7.2	GCAATGCTGACTCCAAACTCT	GGCCTCATTCTATGGCTCA	58.7
8	ATGTGGTTTCACCGTCAACA	AGGCAAGAGGCCAGTCAGTA	64.3
9.1	ATGTATCAAATAGTCAATATGCAA TGT	TCAAAAAGTTGAGGCTTCCAG	65.8
9.2	TGGAAGTGGACAACGAAACA	TTCCAAAACAGAGGCAAGAA	58.7
9.3	TTGCATGGAGGAAACTGTGA	CAGTGTCACCCTGTTTCAGCA	64.3
10	CCTCCAGCAGGAGCTTTTTA	AAAATGTTCTCCCCTTTGACC	60.6
11	AGGGACCTGGGTTTCTGCT	GGGAAGAAATTGCAGTGGTC	64.3
12.1	GCTTGCTCTGGTTGGTCTC	GCGGAACCACTGTGAAAGTT	64.3
12.2	TGACAATGTTAATCTGCAACTGG	AAGCAGGTTCTTCAAATGTTCC	64.3

422 Appendix 1

423





