

1 **Functional and Anatomical Outcomes of Choroidal Neovascularisation**
2 **complicating *BEST1* related retinopathy**

3 **Abbreviated Title:**

4 **Outcomes of CNV in *BEST1* related retinopathy**

5 Kamron N Khan, PhD, FRCOphth¹⁻³

6 Omar A Mahroo, PhD, FRCOphth^{1,2}

7 Farrah Islam, FCPS, FRCS.²

8 Andrew R Webster, MD(Res) , FRCOphth^{1,2}

9 Anthony T Moore, FRCS, FRCOPhth^{1,2,4}

10 Michel Michaelides, MD(Res), FRCOphth^{1,2}

11 1. University College London Institute of Ophthalmology, University College London, London,
12 UK.

13 2. Medical Retina Service, Moorfields Eye Hospital, London, UK.

14 3. Department of Ophthalmology, Leeds Institute of Molecular Medicine, St James' University
15 Hospital, Beckett St, Leeds, UK.

16 4. Ophthalmology Department, University of California San Francisco Medical School, San
17 Francisco, California, USA.

18 Corresponding authors: Kamron Khan and Michel Michaelides at address 1 above. Email:

19 medknk@leeds.ac.uk and michel.michaelides@ucl.ac.uk

20 Grants/ Financial Disclosure: National Institute for Health Research Biomedical Research Centre at
21 Moorfields Eye Hospital National Health Service Foundation Trust and UCL Institute of
22 Ophthalmology (UK; KNK, ARW, ATM, MM), Fight For Sight (UK; ARW, MM, OAM), Moorfields Eye
23 Hospital Special Trustees (UK; MM), the Foundation Fighting Blindness (FFB, USA; ARW, ATM, MM),
24 Retinitis Pigmentosa Fighting Blindness (UK; ARW, ATM, MM), and the Wellcome Trust
25 (099173/Z/12/Z; ARW, MM). Michel Michaelides is supported by an FFB Career Development Award.
26 This research has been funded/supported by the National Institute for Health Research Rare
27 Diseases Translational Research Collaboration (NIHR RD-TRC). The views expressed are those of the

28 author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.”
29

30 Proprietary Interest : None

31 CONFLICT OF INTEREST: No conflicting relationship exists for any of the authors.

32 **Acknowledgements**

33 The authors thank Sarah Hull, University College London, for help in identifying patients.

34

35

36 KEY WORDS

37 Autosomal Recessive Best Retinopathy.

38 Best related Retinopathy.

39 Choroidal neovascularization.

40 Retina.

41 Retinal dystrophy.

42 SUMMARY STATEMENT

43 Choroidal neovascularization is a rare cause of visual loss in patients with Best disease. Its optimal
44 management is unknown. We highlight novel clinical features of disease and present outcome data
45 suggesting that a better outcome might be obtained with anti-VEGF therapy.

46

47

48

49

50

51 **Functional and Anatomical Outcomes of Choroidal Neovascularisation**
52 **complicating *BEST1* related retinopathy**

53 **Abstract**

54 **Purpose:**

55 To describe the presenting features and functional outcomes in a series of patients with choroidal
56 neovascularization (CNVM) complicating *BEST1* related retinopathy (Best Disease, BD and Autosomal
57 Recessive Bestrophinopathy, ARB).

58 **Methods:**

59 Retrospective review of consecutive cases at a tertiary care eye hospital. Patients were identified
60 retrospectively over an 11-year period. Records were reviewed to extract demographic, as well as
61 functional and anatomical outcome data.

62 **Results**

63 14 eyes of 12 patients were identified (11 BD, 1 ARB). Median follow up was 2.8 years (range 0.8 to
64 6). The median age at CNVM discovery was 15.5 years (range 6 to 72). CNVM were active early in the
65 disease course prior to vitellirupture. Seven eyes were treated with intravitreal bevacizumab, 7 eyes
66 were monitored by observation alone. On average patients required a single treatment (median = 1,
67 range 1-10). The median gain in visual acuity (VA) was greater in the treated versus the observed
68 group - 0.46 v 0.17 decimalised units of Snellen Acuity respectively ($p < 0.05$ Mann-Whitney U test).
69 Although a significant reduction in central macular thickness (CMT) was evident in both groups,
70 150 μ m (treated) and 104 μ m (observed), active treatment was not associated with greater thinning
71 than observation ($p > 0.05$ Mann-Whitney U test).

72 **Conclusions**

73 There is a high rate of spontaneous recovery of *BEST1*-related CNVM, and overall we observed a gain
74 in VA associated with a reduction in CMT. Active treatment, here with intravitreal bevacizumab, is
75 associated with better functional outcomes than observation alone.

76 **Introduction**

77 The bestrophinopathies are a spectrum of inherited retinal dystrophies that result from mutation of
78 the *BEST1* gene. The commonest presentation within this group is Best Disease (BD; Vitelliform
79 Macular Dystrophy; OMIM 153700), a macular dystrophy characterised by bilateral accumulation of
80 subretinal yellow material with later eruption into the photoreceptor layer and symptomatic
81 reduction in vision. This form of the disease is most commonly associated with heterozygous
82 missense mutations usually within the first half of the *BEST1* gene.^{1,2} BD is almost always associated
83 with a reduced light rise of the electrooculogram (EOG).³³ The full-field electroretinogram (ffERG) is
84 normal. In contrast, autosomal recessive bestrophinopathy (ARB; OMIM 611809) associated with bi-
85 allelic *BEST1* variants results in a more widespread retinal disease with multifocal accumulation of
86 subretinal deposit, and abnormalities of the ffERG in addition to a reduced EOG light rise.⁴

87 In both BD and ARB, central visual acuity may be affected at any stage, although this usually is
88 associated with either intraretinal fluid (IRF) accumulation, disruption of the photoreceptor layer
89 during the vitelliruptive stage of dominant disease, or later atrophy. Rarely, visual decline may be
90 the result of choroidal neovascular membrane (CNVM) formation. Whilst there are a few case
91 reports and small series suggesting that CNVM can be successfully treated with intravitreal injections
92 of recombinant antibodies directed against vascular endothelial growth factor (VEGF) (Ranibizumab,
93 Bevacizumab), there is no evidence to suggest that outcomes are better than conservative
94 management (observation alone).⁵⁻⁸ Here we report our clinical experience with a cohort of patients
95 with BD and ARB, whose disease has been complicated by CNVM.

96

97

98 **Methods**

99 A retrospective review of the electronic patient record system at Moorfields Eye Hospital was
100 performed with the search terms ‘Best disease’, ‘recessive bestrophinopathy’, ‘choroidal
101 neovascularisation’ and ‘haemorrhage’ covering the time period between 2003 and 2015. The
102 hospital notes were then reviewed both to confirm the diagnosis and document the clinical findings.
103 Only patients with active CNVM were included. CNVM were deemed active if there were two of the
104 following features were present – acute visual deterioration, retinal haemorrhage or exudate,
105 intraretinal fluid, irregular pigment epithelial detachment or evidence of neovascularisation with
106 fluorescein angiography. Presumed inactive CNVM were diagnosed primarily on the basis of
107 subretinal fibrosis and excluded from this study. Patient demographics (including sex and age at
108 CNVM diagnosis) and symptoms were noted. Snellen visual acuity recorded in the clinic was
109 converted into a decimalised value for subsequent analysis. Retinal anatomy was documented with
110 colour fundus photography using a Topcon TRC 50IA retinal camera (Topcon Corporation, Tokyo,
111 Japan) and optical coherence tomography (OCT) using the Spectralis SD-OCT (Heidelberg
112 Engineering, Heidelberg, Germany), with both line and volume scans available for interpretation.
113 Fundus autofluorescence (FAF) images were acquired using the AF mode of the Spectralis SD-OCT
114 using either the 30 or 55 degree lenses. Fundus fluorescein angiography (FFA) was performed using
115 either the Topcon fundus camera or the Spectralis HRA systems.

116 In order to estimate the period prevalence of CNVM in *BEST1* related eye disease we reviewed
117 retinal images of patients’ known to harbour *BEST1* mutations attending between 1.1.2010 and
118 1.1.2015. Only patients with active disease (as defined above) were included. Numerical data are
119 described using median values and interquartile ranges, and compared using non-parametric
120 analysis (Mann-Whitney U test).

121 This study was approved by the local research ethics committee, and all investigations were
122 conducted in accordance with the principles of the Declaration of Helsinki.

123 **Results**

124 *Cohort demographics*

125 Twelve patients were identified over the study period with a clinical diagnosis of either BD (n=11) or
126 ARB (n=1) complicated by active CNVM. Molecular confirmation of the clinical diagnosis was
127 available in 6/12 cases; genetic testing was not performed in two patients (Table 1). For the
128 molecularly unconfirmed cases, confidence in the clinical diagnosis was high as patients presented
129 with at least two classical features of BD (macular phenotype, reduced EOG, dominant family
130 history). Six patients (50%) were male. Two patients had bilateral CNVM, thus data from 14 eyes of
131 twelve patients were available for analysis. The follow up period ranged from 0.8 to 6 years. One
132 patient was diagnosed with BD as a child but only developed signs of CNVM aged 72 years (Patient
133 4). The median age at CNVM discovery was 15.5 years (IQR 13 years). For the two patients with
134 bilateral disease, sequential involvement of the fellow eye was observed within 4 years. Figure 1
135 shows images from the fellow eye of patient 8 to illustrate the natural history of BD uncomplicated
136 by CNVM, for comparison with subsequent figures (Fig. 2-5), which show illustrative images from
137 Patients 2 and 9; salient features will be discussed in the Results and Discussion below.

138 *Premorbid and presenting clinical characteristics*

139 For 11/14 eyes the acuity prior to CNVM discovery was known and documented to be normal in 9/11
140 (median = 20/20, IQR = 0). 3/14 eyes presented with active disease, consequently the prior acuity
141 was unknown. For 2/11 the baseline visual acuity was already reduced. Patient 4 presented aged 72
142 with advanced BD; her results will be presented separately. Patient 2 had suffered with prior
143 neovascular disease in the same eye more than two years previously, accounting for her reduced
144 presenting vision. Visual acuity in the fellow eye at the time of CNVM discovery was similar to the
145 pre-CNVM acuity in affected eyes (median 20/20, IQR = 0), strongly suggesting that both eyes were
146 at a similar stage of disease and that neovascularisation occurs relatively early in the disease course.
147 The median visual acuity at CNVM discovery was 20/60 (0.33, IQR = 0.17), overall representing a
148 moderate reduction from baseline, although for individual patients this varied significantly (range =
149 20/20 to 20/400) (Table 1). The presenting acuity did not appear to be inversely correlated with
150 acuity in the fellow eye. Patient 4 began with acuity of 20/120 right and 20/80 left. Her vision then
151 fell to 20/200 in the right eye as the CNVM became active; 5 years later subretinal scarring resulted
152 in a final acuity of 20/1200. The fellow eye remained at 20/80 throughout the follow up period.

153 Where SD-OCT scans were available prior to CNVM detection (n= 9), pre-existing SRF was present in
154 seven cases (example shown in Fig. 2A). IRF however was never observed. All patients reported a

155 symptomatic reduction in their central vision (n=14 eyes). In all cases haemorrhage was noted at
156 some point in the disease course, and was always subretinal in location and found either inside the
157 boundaries of the yellow vitelliform lesion or at its border. Four patients presented in the month
158 preceding hemorrhage detection with new symptoms of dysmorphopsia. In these cases FFA showed
159 no evidence of vascular leakage. It is however possible that they had active neovascular disease at
160 this time that evaded detection with conventional imaging techniques.

161 *Imaging in active disease*

162 Fundus fluorescein angiography (FFA) was requested in 9/14 cases and available for review. In a
163 minority (3/9) late leakage of undetermined origin was evident. The majority (6/9) however
164 demonstrated staining of the hyperfluorescent subretinal deposit thus masking any subtle changes
165 being further characterised. Indocyanine green angiography performed in one case (Patient 12)
166 showed only masking.

167 Optical coherence tomographic imaging of the retina-RPE-choroidal interface was available for all
168 cases (14 eyes) and demonstrated abnormalities in the four cases that were symptomatic prior to
169 haemorrhage being visualised. The earliest visible changes were at the level of the RPE, with
170 separation from Bruch's membrane due to presumed CNVM (see Figures 2b, 3b, 4e, 5a). The
171 maximal site of RPE elevation was always found in the lower half of the macular lesion (n=14), and
172 often no disturbance was seen in the superior half (see Figure 3A and 3B). Other OCT features
173 observed in patients with CNVM were subretinal fluid (SRF) (n=14), new IRF (n=12), discontinuity in
174 Bruch's membrane (n=7), areas of choroidal excavation (n=4) and presumed photoreceptor outer
175 segment delamination (n=1). At the final follow up visit SRF was still present in all eyes.

176 Fundus autofluorescence imaging was used to identify subretinal deposition, which was
177 hyperautofluorescent in all cases. Areas with SRF exhibited reduced autofluorescence and when
178 haemorrhage was present the normal autofluorescence was masked to a greater extent than by fluid
179 alone. Inactive CNVM associated with fibrosis and organising haemorrhage that had become
180 depigmented may be mistaken for yellow subretinal deposit seen in typical BD. Autofluorescence
181 was useful in differentiating deposit (hyperautofluorescent) from fibrosis and scarring which are
182 both hypoautofluorescent (Figures 4B and 5D,E show autofluorescence images from the same eye
183 before and after CNV development). Rupture of the RPE resulting from vertical extension of a CNVM
184 was again associated with reduced autofluorescence.

185 *Outcomes*

186 As all the patients identified presented with active CNVM after 2009, treatment with intravitreal
187 bevacizumab (1.25mg/0.05ml) was potentially available to all cases. Seven eyes in this series
188 received active treatment. Three of these patients had neovascular disease in their better seeing
189 eye, as fellow eyes were affected by amblyopia (n=2) or prior CNVM (n=2); factors which may have
190 influenced the decision to use an anti-VEGF agent. The majority (5/7) of treated patients were over
191 the age of 18, perhaps reflecting the ease of administering intravitreal therapy in an adult versus
192 paediatric population where sedation or general anaesthesia may be required. Of the seven treated
193 patients, four had a single injection, one received a second injection, one (Patient 12) had a
194 predetermined course of three 'loading' injections and one (Patient 8) received ten intravitreal
195 treatments. The multiple injections required by Patient 8 may represent partial response to this
196 therapy, membrane recurrence, or the inability to correctly identify an endpoint for treatment.
197 Patient 2 was also thought to have developed late CNVM recurrence surrounding a previously
198 inactive disciform scar.

199 As a group, the median change in vision after presenting with an active CNVM until the final follow
200 up visit was a gain of 0.34 decimalised Snellen lines (IQR 0.48), equivalent to a change from 20/60
201 (median presenting acuity) to 20/32. The treated eyes had a median gain in vision of 0.46 (IQR =
202 0.32), whilst eyes monitored by observation alone also gained vision, recording a more modest
203 increase of 0.17 decimalised Snellen acuity (median gain, IQR = 0.39). This difference was found to
204 be significant (Mann-Whitney U test, $p < 0.05$).

205 Central retinal thickening was evident in all cases at CNVM discovery, with a median central 1mm
206 macular thickness of 561 μ m (IQR = 160) (observed) compared to 411 μ m (IQR = 441) (treated)
207 (Mann-Whitney U test, $p > 0.05$). At final follow up this had reduced to a similar extent in both the
208 groups; 150 μ m (IQR = 41) (observed) and 104 μ m (IQR = 240) (treated) (Mann-Whitney U test, $p >$
209 0.05).

210 During a five-year interval from 2010-2015, 107 molecularly confirmed cases of *BEST1* related eye
211 disease were recorded at Moorfields Eye Hospital. Molecular genetic testing is offered as an adjunct
212 to the clinical examination, particularly if there is any clinical doubt regarding diagnosis. Testing
213 would not have been offered, or accepted, in a further unquantifiable cohort of patients. Six of these
214 patients later presented with active CNVM, suggesting a minimum prevalence of 5.6% (6/107) during
215 this period.

216

217 |

218 **Discussion**

219 Choroidal neovascularisation is thought to be a rare complication of *BEST1* related retinopathy;
220 however the exact prevalence is currently unknown.⁹⁻¹³ In this study we have identified 14 eyes from
221 twelve patients who have presented with active choroidal neovascular disease associated with
222 either BD or ARB. Within our own genetic database, this equates to a period prevalence of 5.6%,
223 significantly higher than one may have expected. In the majority of cases, neovascularisation
224 occurred early in the disease course, when visual acuity would otherwise be unaffected. The natural
225 history of these membranes appears to follow a more benign course than those associated with age-
226 related macular degeneration (ARMD), with a median gain of 0.34 decimalised units of Snellen acuity
227 after resolution. Treatment with intravitreal bevacizumab (n=7) was associated with greater visual
228 gain when compared to observation alone (n=7, Mann-Whitney U test p <0.05).

229 Diagnosing CNVM in the context of *BEST1* related retinopathy is complicated by the presence of pre-
230 existing subretinal deposit, which stains during FFA. We suggest additional features that may aid
231 diagnosis or at least heighten clinical suspicion. Typical disease uncomplicated by neovascularisation
232 is associated with subretinal deposit that organises over time, and often is later accompanied by SRF
233 (Figure 1). The residual deposit becomes distributed in a pattern that is primarily influenced by
234 gravity resulting in a predominantly inferior accumulation (the pseudohypopyon stage). Persistence
235 of the dense deposit inferiorly may result in a greater insult to the inferior retina-RPE complex than
236 that in the superior macula. In keeping with this hypothesis, whenever we were able to identify the
237 neovascular complex it was always sited within the inferior half of the vitelliform lesion (Figures 2-5).
238 In no cases were membranes seen to arise from the superior half of the lesion. In most cases the
239 CNVM develop relatively early in the disease course, prior to vitellirruption, as normal acuity had
240 been recorded within the past seven months in 82% (9/11 eyes), suggesting normal central
241 photoreceptor function. As the membranes grow, they breach Bruch's membrane and distort the
242 RPE resulting in localised detachments (Figures 2b, 3b, 4e, 5a). In the absence of CNVM the RPE
243 should otherwise appear flat and apposed to Bruch's membrane (Figure 1). Bruch's membrane is
244 usually not visible on OCT. RPE detachment results in the two structures now being separately
245 resolved (Figures 2b, 3b, 4e), which are presumed to be the RPE and Bruch's membrane. Type 1
246 membranes sit below the RPE and in the earliest stages are not perfused.¹⁴ As they mature and
247 support a blood flow they may leak, just as an occult CNVM associated with AMD would. Serous
248 leakage into the sub-RPE compartment may result in fibrosis and RPE hyperplasia without the
249 appearance of frank haemorrhage.¹⁵ This may account for the fibrotic appearance of the macula in
250 some patients with BD rather than the better defined atrophic maculopathy. If this is the case,

251 CNVM may be a more common complication of BD as both atrophy and fibrosis were recognised as
252 endpoints for this disease when it was originally classified. Should the CNVM breach the RPE
253 becoming a type 2 membrane it can grow within the vitelliform space.¹⁴ Contact with the subretinal
254 surface provides a scaffold for progression, with or without duplication of the RPE. The membrane
255 may bleed into the subretinal as well as sub RPE cavity or leak serous fluid. The presence of
256 definitively new fluid would be hard to detect as SRF is a typical feature of BD in the absence of
257 CNVM, but CNVM activity may additionally result in IRF, not typically seen in BD (Figures 2c, 3a) but
258 present in ARB (Figure 6). As the CNVM contracts it may exert tractional forces on the subretinal
259 surface, and as there is sufficient space within the fluid filled cavity we can occasionally observe a
260 presumed detachment/delamination of the photoreceptor outer segments (present in Figure 3c).
261 Abnormal neovascular networks may also form anastomoses between the retinal and choroidal
262 circulations (Type 3 membranes).

263 It is likely that a recently developed imaging technique, OCT angiography (OCTA), will offer the best
264 method of visualising well perfused CNVMs, as it is minimally influenced by the presence of
265 subretinal deposit unlike FFA (personal observation, unpublished data). It will be interesting to see in
266 these cases if CNVM anatomy as defined by OCTA correlates to visual outcome, as this may help to
267 provide further prognostic information. If it becomes evident that specific subtypes of CNVM are
268 associated with a better prognosis, or are more responsive to treatment, as is the case in ARMD, this
269 additional information will be particularly helpful in the management of paediatric patients, where
270 organising treatment is not as straight forward as for adults.¹⁵

271 Age related CNVM are associated with diffuse thickening of the RPE basal membrane (basal laminar
272 deposit) and secondary dystrophic calcification, whilst pediatric CNVM are not.¹⁶ “Juvenile”
273 membranes are thought to result from a more localised abnormality with a solitary site of subretinal
274 vascular invasion rather than the multifocal vascular breaches that occur with age.¹⁷ This is in
275 keeping with the natural history and prognosis for these membranes being better than for those
276 which occur in ARMD, and may explain why spontaneous regression is reported to be very common
277 in pediatric CNVM.^{18, 19} The initial report of visual outcomes in BD complicated by CNVM monitored
278 by observation alone suggested that recovery could be expected in the majority of cases (10/11 eyes
279 in the initial series) with 9/11 eyes recording a final acuity of better than 20/50.⁹ Smaller, more focal
280 CNVM may also explain their sensitivity to treatment with anti-VEGF agents, as single treatments are
281 often sufficient.^{5-8, 20} Rishi et al have most recently reported their experience treating pediatric
282 CNVM and present data on four patients with BD who were followed up for more than one week.²¹
283 The 3 patients who received treatment showed either an improvement (from 20/200 to 20/120 and

284 20/20) or stabilisation of vision (at 20/30). One patient with inactive disease was followed by
285 observation only and his vision spontaneously improved (20/200 to 20/30), again highlighting the
286 good visual outcome that may be seen with spontaneous regression of CNV in children. Pediatric
287 CNVM may also complicate structural abnormalities (angioid streaks, choroidal osteoma, optic nerve
288 head drusen, trauma and less commonly myopia), intraocular inflammation (presumed ocular
289 histoplasmosis syndrome, multifocal choroiditis, toxoplasmosis) or be idiopathic.²¹ CNVM are also a
290 rare complication of other childhood-onset forms of inherited retinal disease and have been
291 reported to occur with choroideremia,²² North Carolina macular dystrophy,²³ and Stargardt
292 disease.²⁴

293 The visual outcomes presented, especially for the untreated group are perhaps surprisingly good,
294 again highlighting the difference with ARMD. In two cases only one treatment was required,
295 suggesting that the membranes are exquisitely sensitive to anti-VEGF or that the disease is
296 monophasic. Endpoints that are valid when treating CNVM associated with ARMD may not be
297 helpful for membranes occurring in the context of BD. We suggest that as SRF can be expected to
298 both pre- and post-date CNVM discovery/activity its usefulness as a biomarker for choroidal
299 neovascularisation is limited. Similarly reliance on automated measurements of central retinal
300 thickness from SD-OCT scans may be unwise, as SRF will be overrepresented within this
301 measurement. This may account for the difference in anatomical and functional outcomes that were
302 recorded here, as patients with typical CNVM recorded better visual outcomes if they received
303 treatment ($p=0.03$), however their central retinal thickness measurements did not mirror these
304 changes. A more accurate use of SD-OCT data may involve segmentation of the retinal sub-layers,
305 recording measurements between the internal limiting membrane (ILM) and ellipsoid zone (EZ),
306 perhaps more representative of IRF. This parameter may correlate better with changes in vision,
307 although our experience of treating patients with ARMD may suggest that this will not always be the
308 case. For ARB even this technique may have limited utility, as IRF is the norm. IRF may also be seen
309 in a minority of cases of end stage BD, so in the setting of significant RPE disease both SRF and IRF
310 may be expected, even in the absence of CNVM, thereby complicating diagnosis.

311 Surprisingly in one case, haemorrhage secondary to the neovascular membrane did not occur until
312 after the age of 70, whilst all other cases were detected under the age of 25 years of age. A number
313 of explanations are possible. Firstly, CNVM occurrence may be independent of disease stage,
314 although in this series 9/14 eyes recorded a normal acuity within the past seven months, suggesting
315 that the majority of photoreceptors were unaffected consistent with the earliest stages of disease.
316 Secondly, this patient may have suffered with prior neovascular complications, and the detected

317 episode in fact represents a recurrence of disease activity. Finally, the CNVM identified might have
318 occurred independent of the *BEST1* mutation, relating instead to ARMD. As monogenic disorders can
319 show phenotypic overlap with ARMD, and mutations in *BEST1* may be non-penetrant and variably
320 expressed, it is also quite possible that *BEST1*-related retinopathy may masquerade as either
321 neovascular or non-neovascular ARMD.

322 Lastly, it is important to highlight that the retrospective nature of this study carries with it inherent
323 limitations. Under-ascertainment is likely to have occurred, most evident due to the lack of cases
324 identified prior to 2009. Molecular confirmation of the diagnosis was not available for 8/14 cases,
325 although all did report a dominant family history consistent with BD, and in two cases a reduced
326 Arden ratio was additionally recorded. Absence of randomisation when selecting the intervention
327 may have provided a bias towards treating the more severe cases, although by chance the
328 presenting visual acuities appear to be well matched between the two groups (median = 0.32 v 0.34,
329 decimalised Snellen). Visual acuity data may also have been influenced by uncorrected refractive
330 error which was not controlled for, but perhaps equally distributed throughout the two groups.
331 Finally, the wide variation in duration of follow up may influence the final acuity, as the natural
332 history of BD is progression to macular atrophy. No such trend was apparent however.

333 In summary, we present the largest case series to date of CNVM complicating BD and ARB. In this
334 non-randomised retrospective series we have identified that these membranes have a high rate of
335 spontaneous resolution, and additional visual gains may be obtained with the use of intravitreal anti-
336 VEGF therapy. In the vast majority of cases CNVM should be considered as a relatively early potential
337 complication of BD. Occasionally this rare complication may recur. We also highlight novel OCT
338 features seen in both early and late neovascular disease that will facilitate identification of these
339 lesions. Lastly, we suggest that CNVM may be a potentially under-recognised complication of *BEST1*-
340 related retinopathy, and the advent of novel imaging techniques may help to prove this.

341

342

343 **REFERENCES**

- 344 1. Petrukhin K et al. Identification of the gene responsible for Best macular dystrophy. *Nature*
345 *genetics* 1998; 19:241-247.
- 346 2. Marquardt A et al. Mutations in a novel gene, VMD2, encoding a protein of unknown
347 properties cause juvenile-onset vitelliform macular dystrophy (Best's disease). *Human molecular*
348 *genetics* 1998; 7:1517-1525.
- 349 3. Deutman AF. Electro-oculography in families with vitelliform dystrophy of the fovea.
350 Detection of the carrier state. *Archives of ophthalmology (Chicago, Ill : 1960)* 1969; 81:305-316.
- 351 4. Burgess R et al. Biallelic mutation of BEST1 causes a distinct retinopathy in humans.
352 *American journal of human genetics* 2008; 82:19-31.
- 353 5. Chhablani J and Jalali S. Intravitreal bevacizumab for choroidal neovascularization secondary
354 to Best vitelliform macular dystrophy in a 6-year-old child. *European journal of ophthalmology* 2012;
355 22:677-679.
- 356 6. Mandal S et al. Intravitreal bevacizumab in choroidal neovascularization associated with
357 Best's vitelliform dystrophy. *Indian journal of ophthalmology* 2011; 59:262-263.
- 358 7. Cennamo G et al. Functional and anatomic changes in bilateral choroidal neovascularization
359 associated with vitelliform macular dystrophy after intravitreal bevacizumab. *Journal of ocular*
360 *pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and*
361 *Therapeutics* 2012; 28:643-646.
- 362 8. Hussain RN et al. Use of Intravitreal Bevacizumab in a 9-Year-Old Child with Choroidal
363 Neovascularization Associated with Autosomal Recessive Bestrophinopathy. *Ophthalmic genetics*
364 2015; 36:265-269.
- 365 9. Chung MM et al. Visual outcome following subretinal hemorrhage in Best disease. *Retina*
366 (Philadelphia, Pa) 2001; 21:575-580.
- 367 10. Iannaccone A et al. Autosomal recessive best vitelliform macular dystrophy: report of a
368 family and management of early-onset neovascular complications. *Archives of ophthalmology*
369 (Chicago, Ill : 1960) 2011; 129:211-217.
- 370 11. Madhusudhan S, Hussain A and Sahni JN. Value of anti-VEGF treatment in choroidal
371 neovascularization associated with autosomal recessive bestrophinopathy. *Digital journal of*
372 *ophthalmology : DJO / sponsored by Massachusetts Eye and Ear Infirmary* 2013; 19:59-63.
- 373 12. Alisa-Victoria K et al. Choroidal neovascularization secondary to Best's vitelliform macular
374 dystrophy in two siblings of a Malay family. *Clinical ophthalmology (Auckland, NZ)* 2014; 8:537-542.
- 375 13. Frennesson CI, Wadelius C and Nilsson SE. Best vitelliform macular dystrophy in a Swedish
376 family: genetic analysis and a seven-year follow-up of photodynamic treatment of a young boy with
377 choroidal neovascularization. *Acta ophthalmologica* 2014; 92:238-242.
- 378 14. Vander JF, Morgan CM and Schatz H. Growth rate of subretinal neovascularization in age-
379 related macular degeneration. *Ophthalmology* 1989; 96:1422-1426; discussion 1426-1429.
- 380 15. Polito A et al. The natural history of occult choroidal neovascularisation associated with age-
381 related macular degeneration. A systematic review. *Annals of the Academy of Medicine, Singapore*
382 2006; 35:145-150.
- 383 16. Spraul CW and Grossniklaus HE. Characteristics of Drusen and Bruch's membrane in
384 postmortem eyes with age-related macular degeneration. *Archives of ophthalmology (Chicago, Ill :*
385 *1960)* 1997; 115:267-273.
- 386 17. Melberg NS, Thomas MA and Burgess DB. The surgical removal of subfoveal choroidal
387 neovascularization. Ingrowth site as a predictor of visual outcome. *Retina (Philadelphia, Pa)* 1996;
388 16:190-195.
- 389 18. Goshorn EB et al. Subretinal neovascularization in children and adolescents. *Journal of*
390 *pediatric ophthalmology and strabismus* 1995; 32:178-182.
- 391 19. Wilson ME and Mazur DO. Choroidal neovascularization in children: report of five cases and
392 literature review. *Journal of pediatric ophthalmology and strabismus* 1988; 25:23-29.

393 20. Leu J, Schrage NF and Degenring RF. Choroidal neovascularisation secondary to Best's
394 disease in a 13-year-old boy treated by intravitreal bevacizumab. Graefe's archive for clinical and
395 experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle
396 Ophthalmologie 2007; 245:1723-1725.

397 21. Rishi P et al. Choroidal neovascularization in 36 eyes of children and adolescents. Eye
398 (London, England) 2013; 27:1158-1168.

399 22. Endo K, Yuzawa M and Ohba N. Choroideremia associated with subretinal neovascular
400 membrane. Acta ophthalmologica Scandinavica 2000; 78:483-486.

401 23. Rhee DY et al. Subfoveal choroidal neovascularization in a 3-year-old child with North
402 Carolina macular dystrophy. Journal of AAPOS : the official publication of the American Association
403 for Pediatric Ophthalmology and Strabismus / American Association for Pediatric Ophthalmology
404 and Strabismus 2007; 11:614-615.

405 24. Klein R et al. Subretinal neovascularization associated with fundus flavimaculatus. Archives
406 of ophthalmology (Chicago, Ill : 1960) 1978; 96:2054-2057.

407

408

409

410

411

412

413

414

415

416

417

418

419

420

421 **Tables**

422 **Table 1. Summary of patient characteristics.**

Patient.	ID.	BEST1 variant	Follow up (years)	Eye with CNVM	Age at CNVM discovery	Treatment given? (Number)	Pre-CNVM VA if known (decimalised Snellen)	Presentation VA (decimalised Snellen)	Final Visit VA (decimalised Snellen)
1	29774	p.Arg356X ; p.Ile201Thr	5	OS	12	No	1	0.4	0.95
2	31575	p.Arg218Cys	1.5	OD	9	Yes (1)	0.43*	0.22	0.5
3	1264937	Not known	3	OD	10	No	1	0.05	0.05
4	12171	p.Phe298Val	6	OD	72	No	0.14	0.1	0.016
5	1597719	Not known	2.5	OD	6	No	Not known	0.32	0.66
			3	OS	6			0.2	0.25
6	31286	Not known	3	OS	13	No	1	0.46	0.63
7	29283	p.Ser16Phe	1.5	OS	20	Yes (1)	Not known	0.34	0.8
8	29668	p.Arg105Gly	4 (6)	OD	24	Yes (10)	1	0.66	1 [^] (0.66) [#]
9	29781	p.Phe298Val	2	OD	28	Yes (2)	1	0.25,	1
			5	OS	25	No	1	0.1	0.66
10	1787207	Not known	1	OD	18	Yes (1)	1	0.34	1
11	1799554	Not known	0.8	OD	10	Yes (1)	1	0.19	0.8
12	2172661	Not known	0.8	OD	19	Yes (3)	1	0.25	1

423

424 * prior CNVM in this eye hence reduced baseline vision

425 [^]VA when considered stable after 4th and 8th treatment

426 [#]VA at final follow up 6 years later

427

428 **Figure Legends**

429 Figure 1. Serial images one year apart from the left eye of Patient 8, showing organisation of the
430 pseudo-vitelliform lesion. Left-hand panels show infrared reflectance images, and right-hand panels
431 show corresponding spectral domain OCT scans taken in the same location. *A*, OCT image shows the
432 subretinal deposit or fluid lies in a mound below a largely intact, but irregular outer retinal ellipsoid
433 line. *B*, Image taken one year later shows greater irregularity of the ellipsoid line with some areas of
434 focal hyperreflectivity in the outer nuclear layer.. *C*, Further images taken one year later show
435 approximation between retina and RPE close to the location of the previous hyperreflective areas,
436 likely to be the area of subsequent scarring. At all time points, the RPE lies flat against Bruch's
437 membrane (itself not visible) with no signs of CNVM.

438 Figure 2. Images from the right eye of Patient 9. *A*, Prior to CNVM development, the RPE lies flat with
439 a shallow foveal detachment. *B*, As the CNVM develops, an irregular RPE elevation becomes evident
440 (white arrow), beneath which Bruch's membrane is now visible. *C*, Signs of active CNVM leakage
441 manifest, with intraretinal fluid, continued presence of subretinal fluid and sub-RPE hemorrhage.
442 This was treated with intravitreal bevacizumab (two injections). *D* and *E*, OCT scans at a later time
443 point showing chronic subretinal fluid superiorly (*D*) and atrophic scarring within the lesion (*E*).

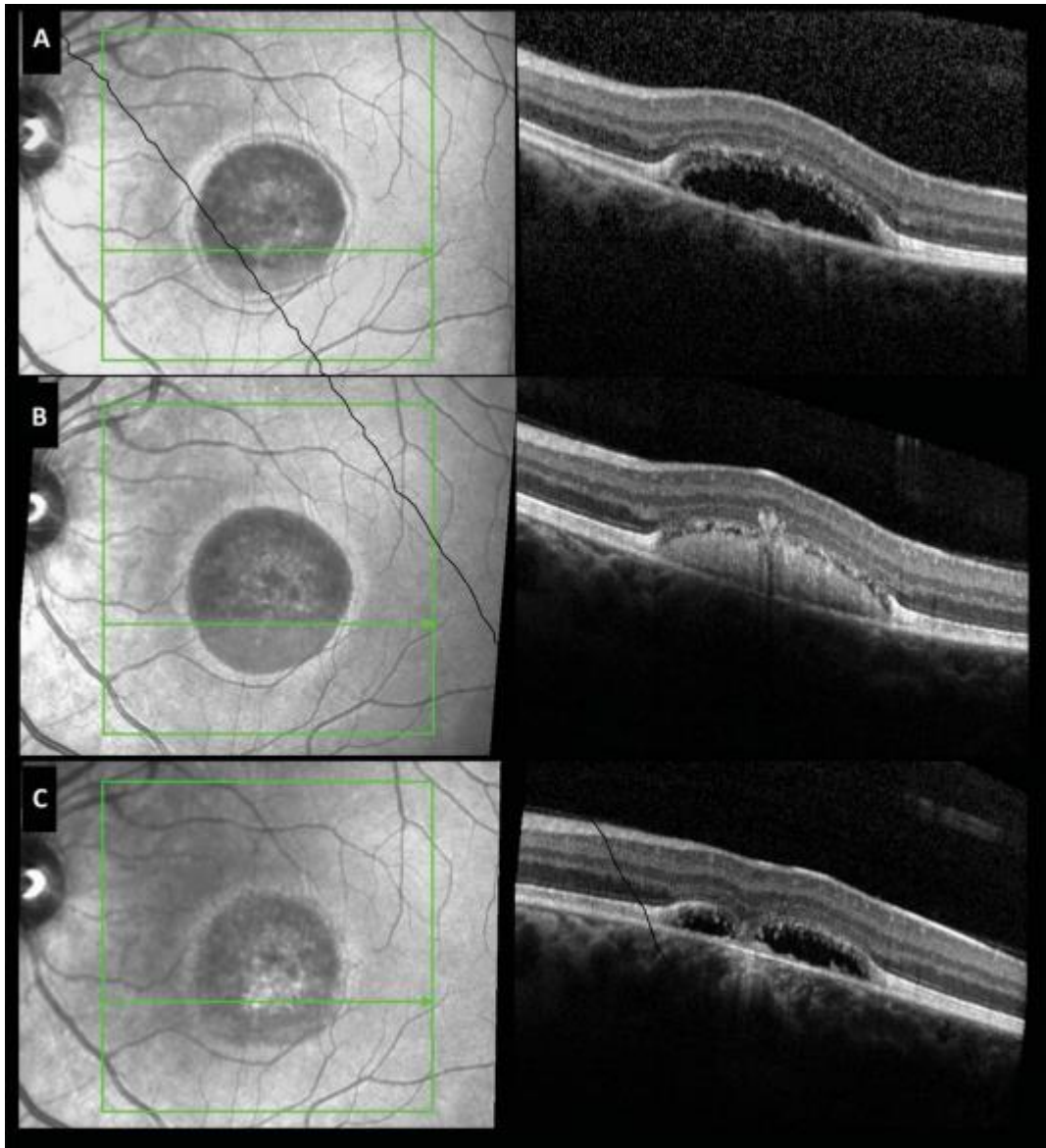
444 Figure 3. Images from Patient 2 with active neovascular disease in the left eye. *A*, OCT scan taken
445 through the superior part of the lesion demonstrates subretinal fluid (between the RPE and the
446 photoreceptor outer segments), cystic expansion of the ONL so that it merges with the OPL,
447 highlighting fluid probably accumulating between the ONL 'proper' and the axonal component of
448 this layer (Henle's layer) with the OPL band representing the dendritic connections between the
449 photoreceptors and bipolar/horizontal cells. There is also microcystic oedema within the inner
450 nuclear layer. *B*, Scanning at a more inferior location identifies a focal hemorrhagic RPE elevation
451 (arrow), blood within the subretinal space and a small additional bright layer between the subretinal
452 blood and the photoreceptor outer segments themselves immediately below the ellipsoid zone. *C*,
453 OCT scan at a similar location 3 months later. The additional highly reflective layer is now absent.
454 The subretinal space below now contains a broad zig-zag shaped line possibly consistent with
455 delaminating photoreceptor outer segments. *D*, Eight months later the ellipsoid layer is not clearly
456 visible at this same location. *E*, Inferiorly, there is a hypertrophic outer retinal scar and persistence of
457 subretinal fluid.

458 Figure 4. Images from the left eye of Patient 9 prior to CNVM development. *A*, Color fundus
459 photograph. *B*, Short wavelength fundus autofluorescence (FAF) image showing
460 hyperautofluorescence of the subretinal deposit. *C*, Fundus fluorescein angiogram (FFA) at 11 s after
461 dye injection. *D*, FFA at 2 min showing staining of the subretinal deposit without clear evidence of
462 active leakage. *E*, OCT image obtained at the same visit.

463 Figure 5. Subsequent images from the left eye of Patient 9. *A*, *B*, *C*, OCT scans taken 2 months later
464 when the patient developed further symptoms, showing elevation of the RPE with possible breach of
465 this layer (arrow in *A*), haemorrhage and inferiorly disruption of the photoreceptor outer segments,
466 possibly resulting from subretinal fluid. *D*, FAF image shows that this is associated with loss of short
467 wavelength autofluorescence within the lesion and a reduction in autofluorescence inferior to the
468 lesion (associated with photoreceptor disruption). This eye did not undergo treatment. *E*, FAF image
469 4 years later showing that the inferior hypoautofluorescence is maintained. *F*, OCT scan at the same
470 location suggests some restoration in outer retinal architecture. *G*, OCT through the lesion at the
471 same visit.

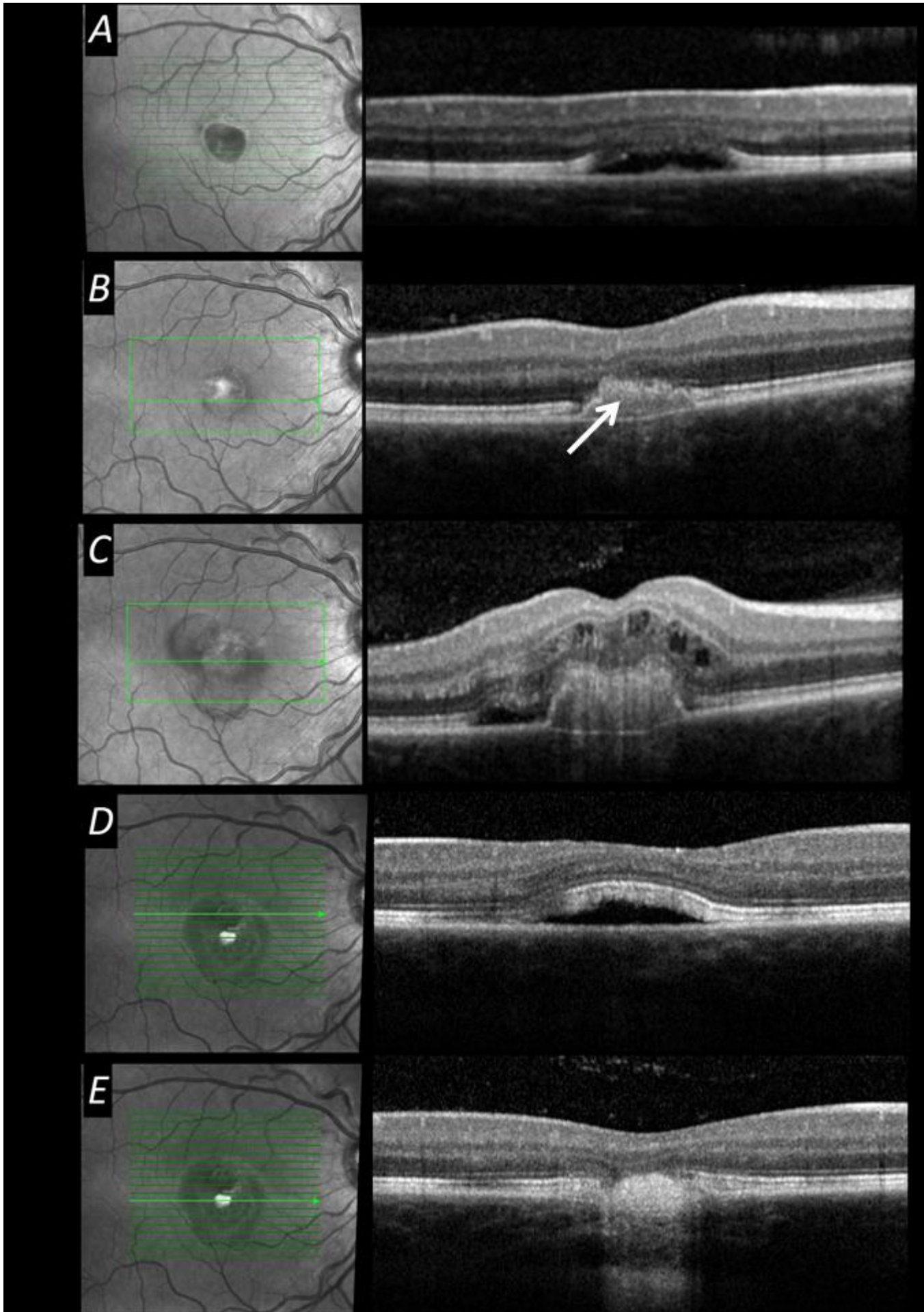
472 Figure 6. Images from a patient diagnosed with ARB showing subretinal and intraretinal fluid in the
473 absence of CNVM. *A* and *B*, OCT scans through the fovea of right and left eye respectively. *C* and *D*,
474 OCT scans through locations inferior to the fovea from right and left eye respectively.

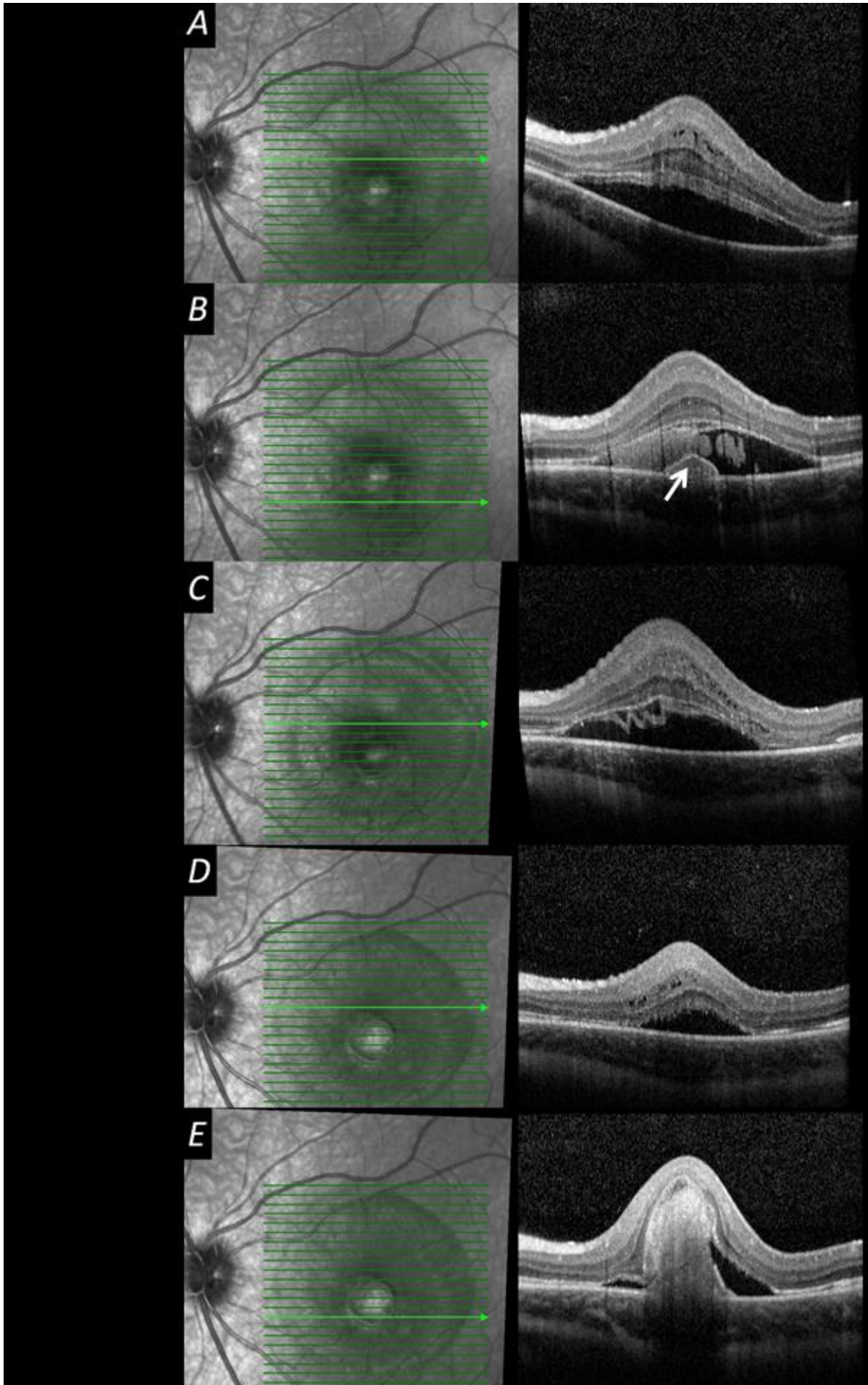
475

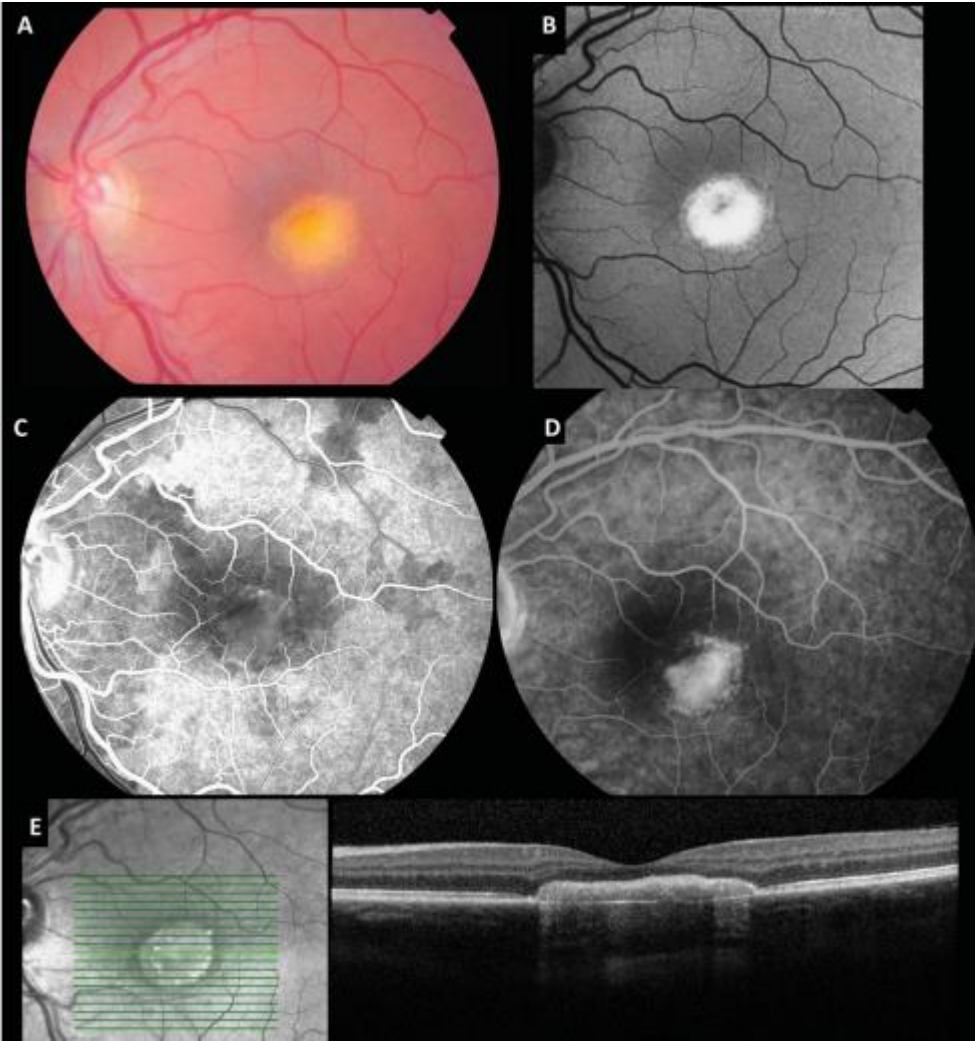


476

477

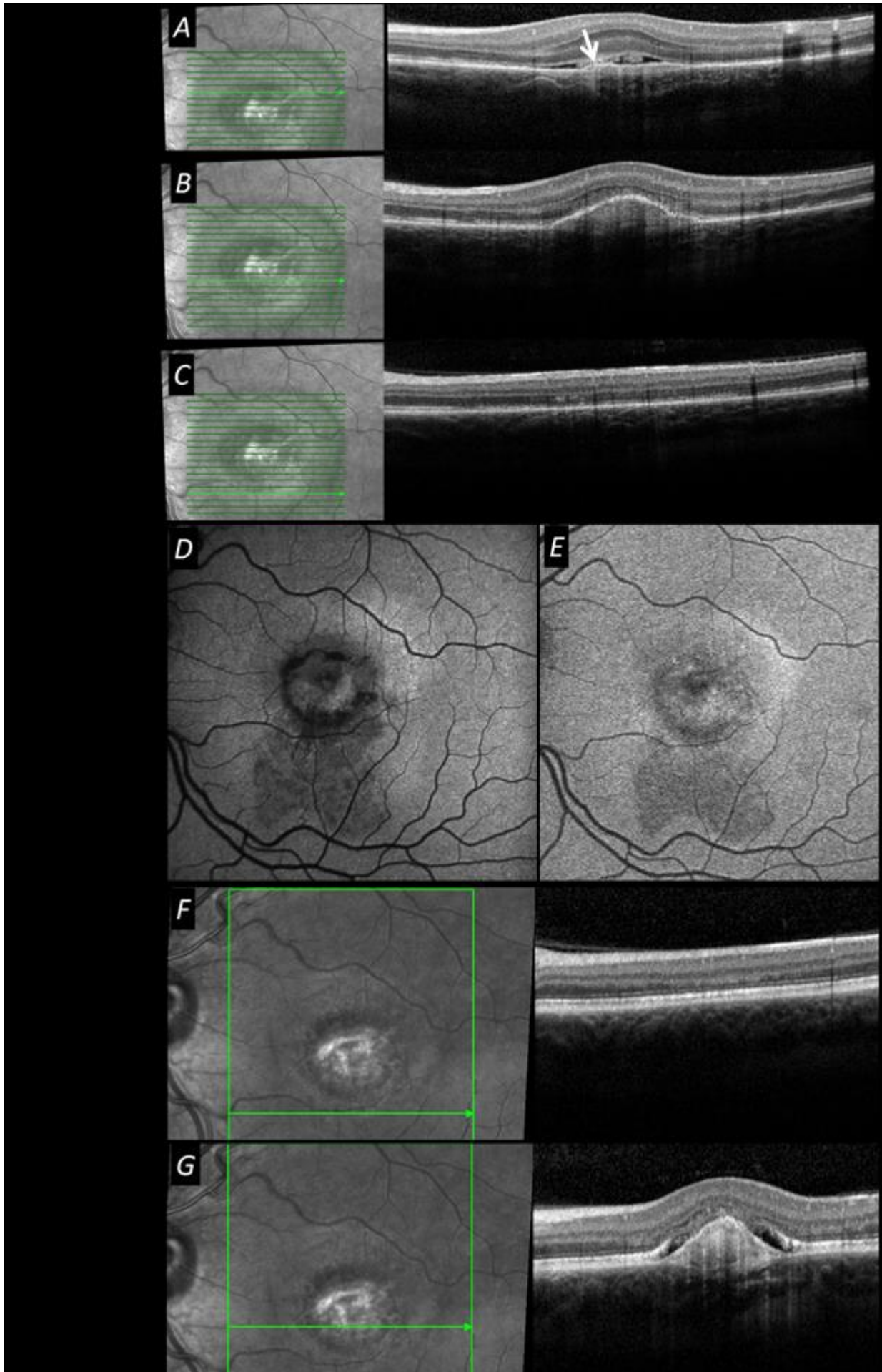


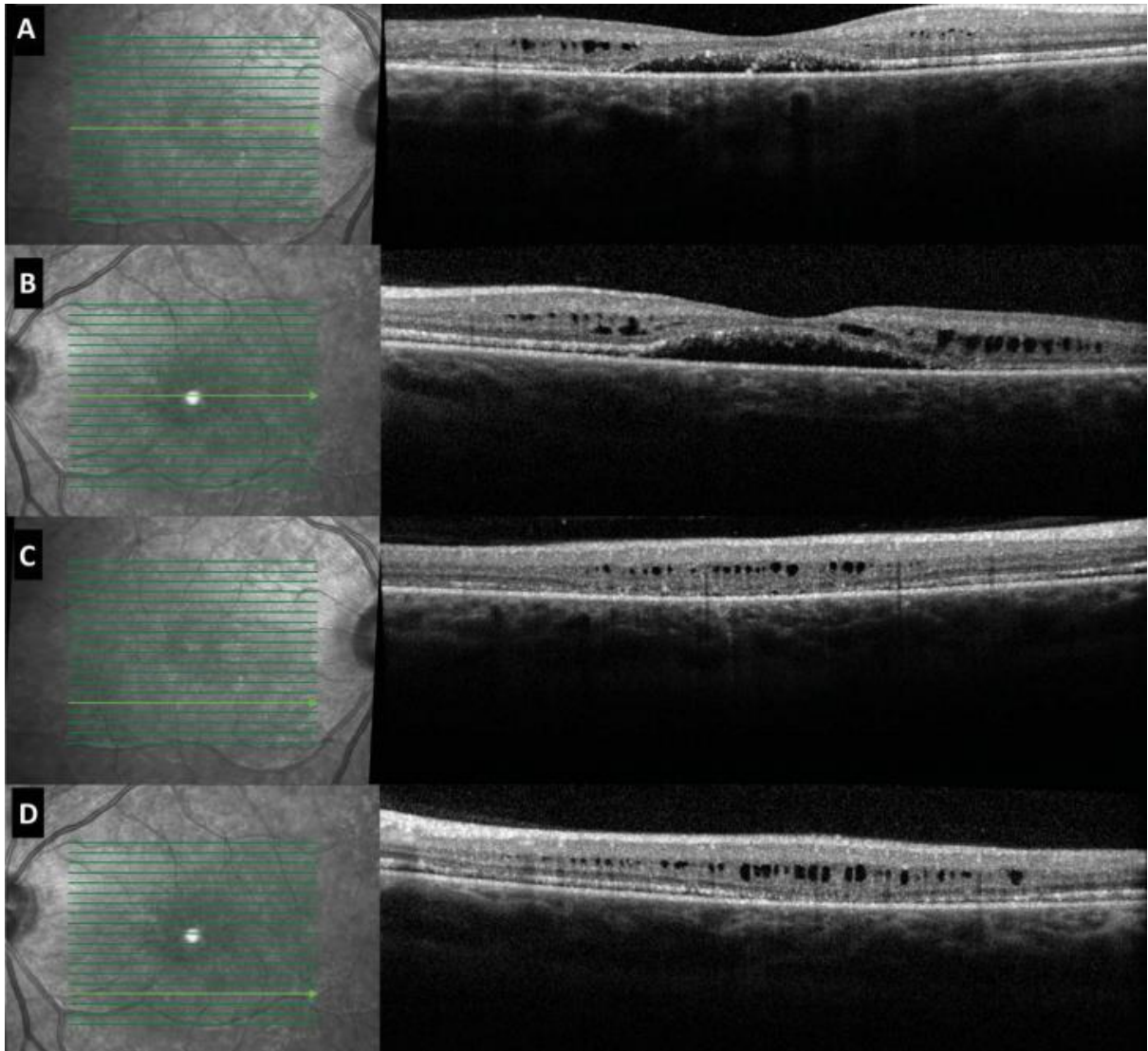




480

481





483