Bietti Crystalline Dystrophy:

Phenotypic Presentation and Inter-Eye Symmetry

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Background

- Bietti Crystalline Dystrophy (BCD) is a rare blinding inherited retinal disease (IRD) for which there is currently no cure.
- BCD is an ideal candidate for retinal gene therapy because:
  - It is a single gene disorder caused by mutations in *CYP4V2*,[1]
  - Characteristic fundus appearance comprising a multitude of small, glistening, yellow-white crystals dispersed across the posterior pole facilitates patient identification.[2]
- However, knowledge on between-eye symmetry in BCD is lacking.
- Clarifying this aspect of disease phenotype would aid:
  - Counselling patients on disease progression, and
  - Designing clinical trials that may use one eye as an untreated control.
- Hence, we set out to examine the between-eye symmetry in a cohort of Australian and New Zealand BCD patients.

Aims

To identify novel anatomical biomarkers of between-eye symmetry, disease progression, and severity, through:

- Quantitatively describing characteristic colour fundus photograph (CFP), fundus autofluorescence (FAF), and optical coherence tomography (OCT) imaging markers among individuals with genetically-confirmed BCD, and
- Investigating correlations between these biomarkers.

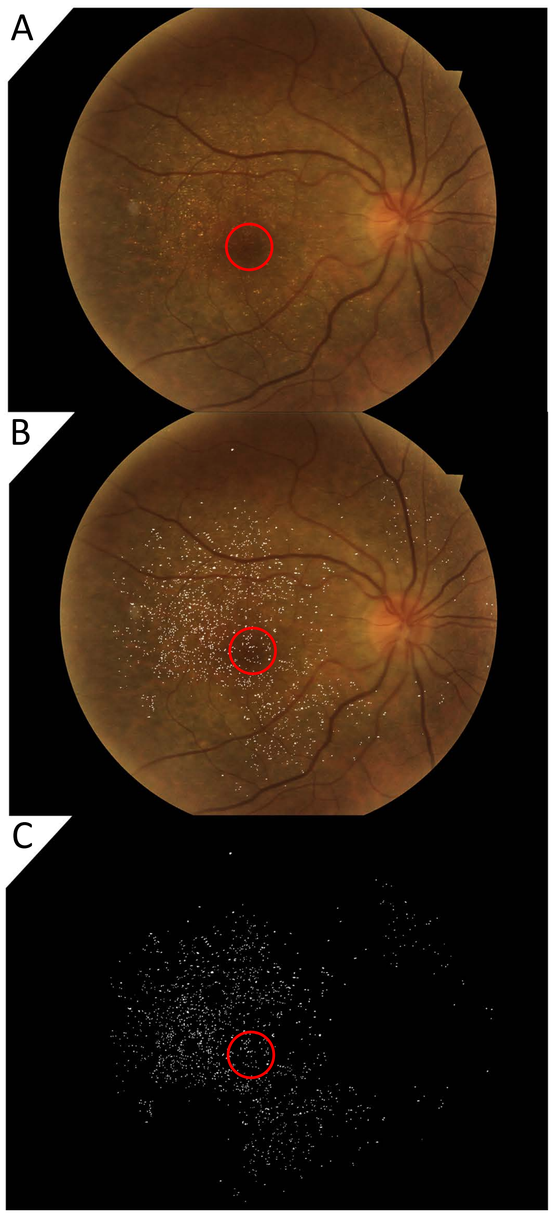

Figure 1: Manual crystal count in the fovea (red circle) and the fundus.
The fovea was taken as a circle of diameter 1 mm. Foveal location was determined visually with optical coherence tomography guidance. Foveal crystal count was then determined manually. Crystals within a 30° fundus image **(A)** were manually traced and shaded in white **(B)**. Crystals were subsequently extracted as a black and white crystal mask **(C)**, from which cumulative fundus crystal count and total crystal area were quantified.

Methods

- Prospective and retrospective data from comprehensive multimodal clinical examinations of 13 Australian and New Zealand participants with confirmed biallelic *CYP4V2* mutations and a characteristic BCD fundus appearance were studied.
- Crystals visible on CFPs were manually counted and superimposed onto aligned FAF imaging.
- Fundus crystal distribution and phenotypic association with areas of absent autofluorescence (absent-AF) were then analysed.
- Spearman's correlation coefficients (ρ), intraclass correlation coefficients (ICCs), and Bland-Altman plots were used to quantify symmetry of functional and imaging parameters between eyes.
  - We analysed crystal count and area on CFP, absent-AF area on FAF, optical coherence tomography (OCT) markers such as foveal thickness and volume, and MAIA microperimetry threshold sensitivities, and best corrected visual acuity (BCVA).

Results

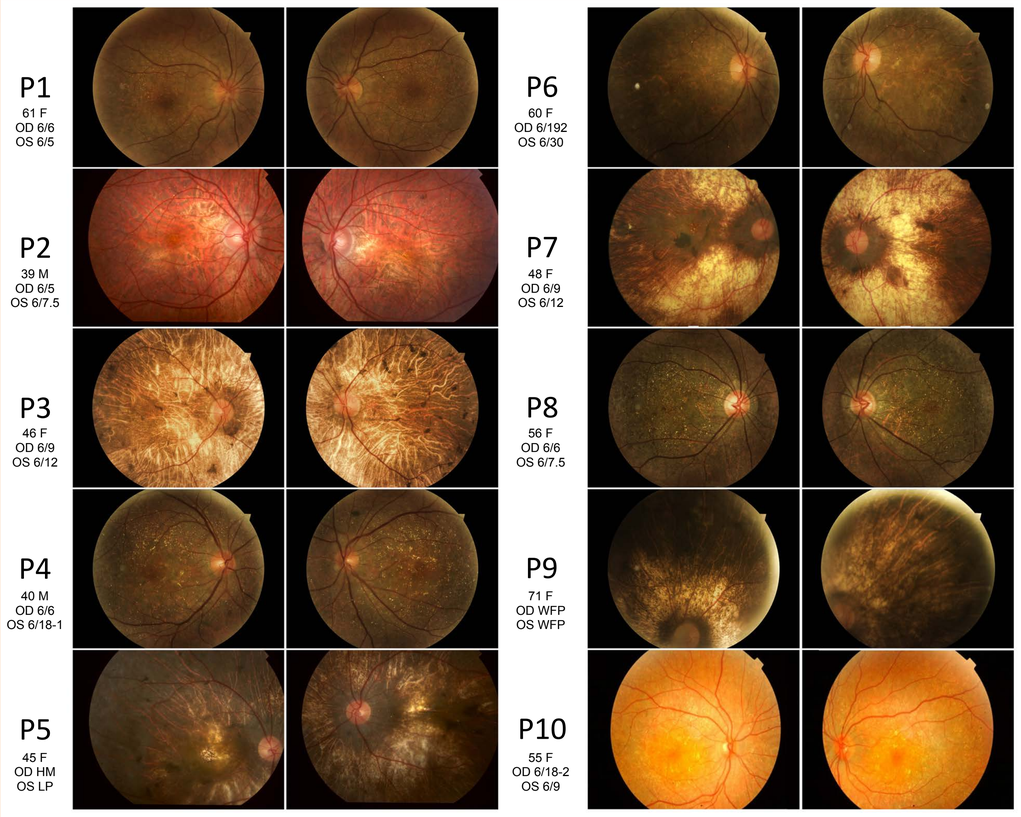

Figure 2: BCD colour fundus photographs (CFP). CFPs demonstrate the variable extent of small, glistening, yellow-white crystalline deposits dispersed throughout the posterior pole in participants with BCD. Also note the variation in chorioretinal atrophy.

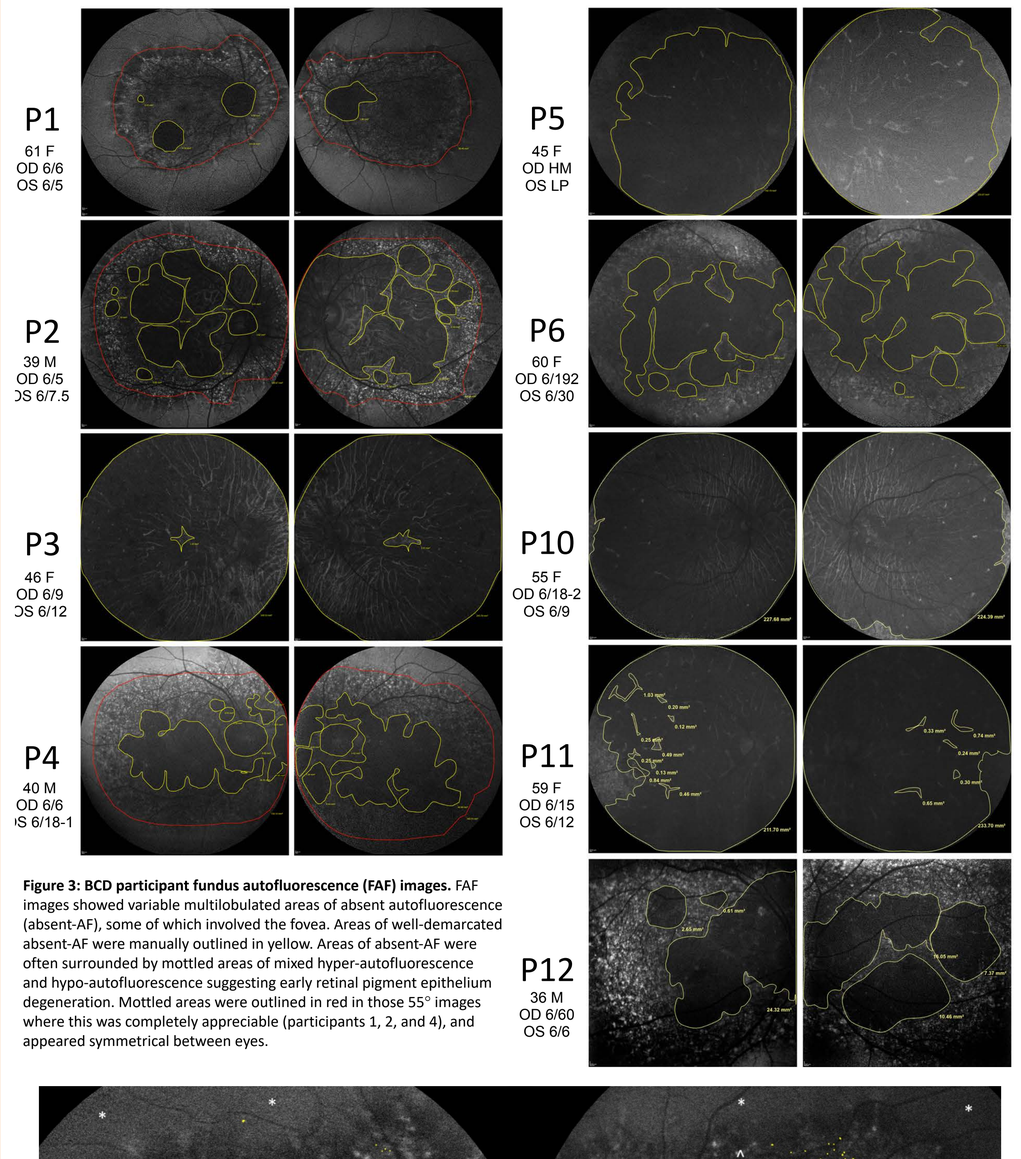

Figure 3: BCD participant fundus autofluorescence (FAF) images. FAF images showed variable multilobulated areas of absent autofluorescence (absent-AF), some of which involved the fovea. Areas of well-demarcated absent-AF were manually outlined in yellow. Areas of absent-AF were often surrounded by mottled areas of mixed hyper-autofluorescence and hypo-autofluorescence suggesting early retinal pigment epithelium degeneration. Mottled areas were outlined in red in those 55° images where this was completely appreciable (participants 1, 2, and 4), and appeared symmetrical between eyes.

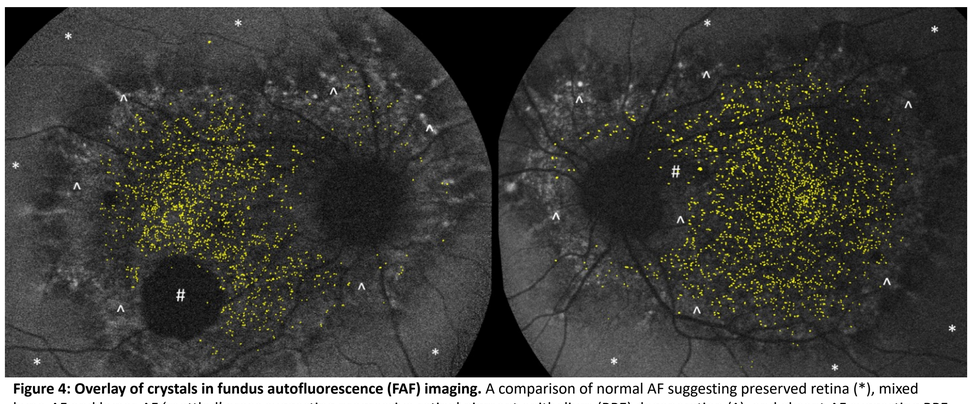

Figure 4: Overlay of crystals in fundus autofluorescence (FAF) imaging. A comparison of normal AF suggesting preserved retina (*), mixed hypo-AF and hyper-AF 'mottled' areas suggesting progressive retinal pigment epithelium (RPE) degeneration (^), and absent-AF suggesting RPE loss (#) in a BCD participant (P1). The yellow dots represent overlayed crystals (manually traced from the corresponding CFP).

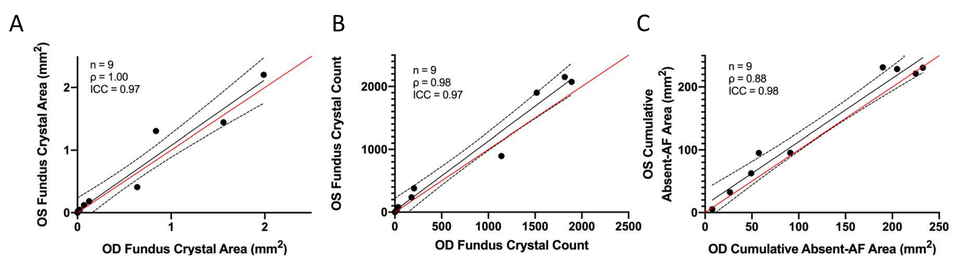

Figure 5: Selected inter-eye correlations in BCD participants, for fundus crystal area (A), fundus crystal count (B), cumulative absent-AF area (C). The black solid lines are the lines of best fit. The black dashed lines are the 95% confidence intervals. The red solid lines are the lines of identity.

Table 1: Population Summary Statistics and Inter-Eye Correlation
Values are n (%), median (IQR), or estimate (95% Confidence Interval (CI)).
Inter-eye correlation analyses in order of decreasing Spearman's ρ. High symmetry (green), moderate symmetry (yellow), low symmetry (red).

Factor	n	Summary			
Participants	13				
Gender					
Male	4	31%			
Female	9	69%			
East Asian Ancestry					
Yes	5	38%			
No	8	62%			
Age (years)	13	48 (40-60)			
		OD	**OS**	**Spearman's ρ (95% CI)**	**ICC (95% CI)**
Fundus Crystal Area (mm²)	9	0.12 (0.01-1.20)	0.18 (0.04-1.37)	1.00 (1.00, 1.00)	0.97 (0.88, 0.99)
Fundus Crystal Count	9	205 (28-1668)	381 (60-1988)	0.98 (0.92, 1.00)	0.97 (0.89, 0.99)
Cumulative absent-AF area (mm²)	9	91.05 (37.93-214.96)	95.24 (47.53-229.67)	0.88 (0.53, 0.98)	0.98 (0.90, 0.99)
Foveal Crystal Area (mm²)	10	0.01 (0.00-0.02)	0.02 (0.00-0.05)	0.81 (0.24,0.94)	0.37 (-0.27,0.79)
Average foveal thickness (µm)	10	318 (272-347)	341 (293-407)	0.76 (0.24,0.94)	0.39 (-0.25,0.80)
Average foveal volume (mm³)	10	0.25 (0.22-0.28)	0.27 (0.23-0.32)	0.73 (0.18, 0.93)	0.85 (0.53, 0.96)
Foveal Crystal Count	10	30 (0-56)	22.5 (10-63)	0.68 (0.08, 0.92)	0.52 (-0.09, 0.85)
BCVA (logMAR units)	13	0.2 (0.1-1.3)	0.3 (0.1-0.6)	0.51 (-0.06, 0.83)	0.94 (0.83, 0.98)
Central foveal thickness (µm)	10	277 (228-323)	313 (244-360)	0.49 (-0.20, 0.86)	0.50 (-0.12, 0.84)
MAIA average macular sensitivity (dB)	8	4.6 (0.2-8.9)	2.8 (1.3-8.6)	0.48 (-0.34, 0.88)	0.86 (0.48, 0.97)
MAIA central foveal sensitivity (dB)	8	11.8 (2.0-22.4)	14.0 (10.3-20.6)	0.47 (-0.35, 0.88)	0.34 (-0.39, 0.82)

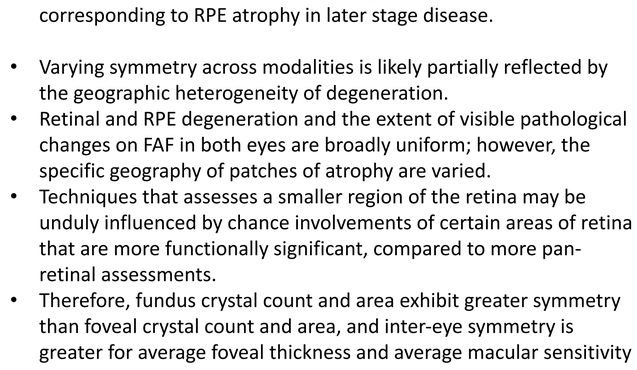

Figure 6: Within eye comparisons of crystal density in BCD participants. (A) Crystal density displayed a pattern of reduction with increasing distance from the fovea. **(B)** Crystal density was reduced at the peripapillary area compared with the 30° fundus. **(C)** Crystal density was reduced in absent-AF regions compared with mottled regions. The horizontal bar represents the median in all panels.

Discussion

- Using a novel approach to quantifying fundus crystals in BCD, we present the first quantification of retinal/retinal pigment epithelium (RPE) crystal count, crystal area, crystal density, and RPE atrophy in BCD.
- Inter-eye symmetry is strong as measured by fundus crystal area and number and cumulative absent-AF area.
- Crystal density decreases with increasing distance away from the fovea, at the peripapillary area, and in absent-AF areas compared to mottled regions.
- Crystal density is low in regions of complete RPE atrophy and in areas of peripheral healthy retina, suggesting a time course of crystal appearance in early to mid-stage disease, and involution corresponding to RPE atrophy in later stage disease.

- Varying symmetry across modalities is likely partially reflected by the geographic heterogeneity of degeneration.
- Retinal and RPE degeneration and the extent of visible pathological changes on FAF in both eyes are broadly uniform; however, the specific geography of patches of atrophy are varied.
- Techniques that assesses a smaller region of the retina may be unduly influenced by chance involvements of certain areas of retina that are more functionally significant, compared to more pan-retinal assessments.
- Therefore, fundus crystal count and area exhibit greater symmetry than foveal crystal count and area, and inter-eye symmetry is greater for average foveal thickness and average macular sensitivity compared to central foveal thickness and sensitivity, respectively.

This study demonstrated strong inter-eye symmetry measured by fundus crystal area, fundus crystal number, and absent-AF area. This may influence the choice of outcome measures for future therapeutic trials for BCD, and provides valuable clinical information for ophthalmologists involved in the care and counselling of BCD patients.

Bibliography

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