Characterization of Retinal Structure in *CNGB3*-associated Achromatopsia

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Purpose:
To investigate retinal structure in *CNGB3*-associated achromatopsia (ACHM), using split detection adaptive optics scanning light ophthalmoscopy (AOSLO)[1] and spectral-domain optical coherence tomography (SD-OCT).

Methods:
Twenty-two ACHM patients underwent ocular examination, SD-OCT and AOSLO imaging. The OCT scans were used for grading the appearance of the fovea and measuring the outer nuclear thickness (ONL). The interpretable split detection AO images were used to quantify peak foveal cone densities (PFD) and inter-cell distance (ICD). The mean and standard deviation (SD) of ICD were used to quantify the coefficient of variation (CV).

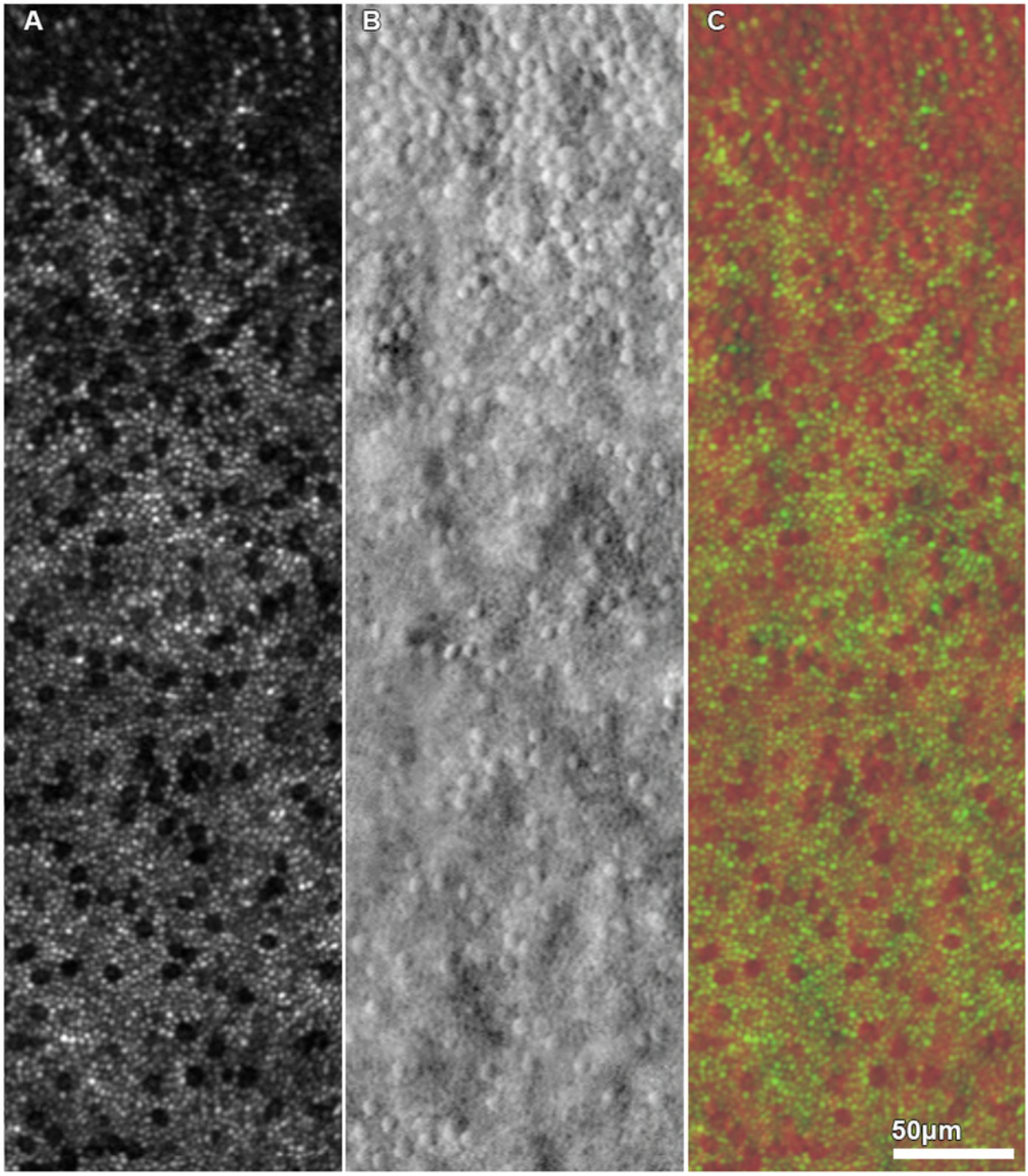
Results:
The mean (range, ±SD) age was 23 years (5-41, ±12.5). Mean (range, ±SD) best-corrected visual acuity (BCVA) was 0.97 LogMAR (0.60-1.52, ±0.21). SD-OCT disruption/loss of the foveal ellipsoid zone (EZ) was graded[2] as; Grade I (continuous ISe): three (13%), II (ISe disrupted): ten (46%), III (ISe absent): one (5%), IV (presence of a hyporeflective zone): seven (32%) and Grade V (retinal atrophy): one (5%) patient. In all the subjects the grade was the same in both eyes. Thirteen patients (59 %) have a variable degree of foveal hypoplasia. The mean (range, ±SD) outer nuclear thickness for all 22 subjects was 66.47µm (37.35-92.45µm, ±16.89). The mean (±SD) ONL thickness for: Grade II was 76.44µm (±11.14) and for Grade IV was 51.52µm (± 21.52). AOSLO images were interpretable in 9 of 22 subjects. Mean (range, ±SD) PFD was 20,133 cones/mm^2 (3,652-50,909, ± 13,940) and the CV (mean ± SD) was 0.25 ± 0.05. No statistically significant correlation was observed between PFD and age (r=-0.17, p=0.67, Pearson Correlation Coefficient).

Conclusions:

The cone mosaic in patients harbouring *CNGB3* mutations was irregular, variably disrupted and with significantly lower peak foveal densities than normal. The ONL thickness was

significantly lower than healthy controls, in keeping with previous reports for *CNGB3* ACHM. The CNGB3-ACHM subjects had a significantly thinner ONL than unaffected subjects, in keeping with previous reports for CNGB3-ACHM.[3] Patients with ACHM should be assessed on an individual basis to determine their potential/remnant cones, for patient selection in upcoming and/or on-going clinical trials.

1. Scoles D, Sulai YN, Langlo CS, et al. In vivo imaging of human cone photoreceptor inner segments. *Investigative ophthalmology & visual science* 2014;55:4244-4251.
2. Sundaram V, Wilde C, Aboshiha J, et al. Retinal structure and function in achromatopsia: implications for gene therapy. *Ophthalmology* 2014;121:234-245.
3. Langlo CS, Patterson EJ, Higgins BP, et al. Residual Foveal Cone Structure in CNGB3-Associated Achromatopsia. *Investigative ophthalmology & visual science* 2016;57:3984-3995.

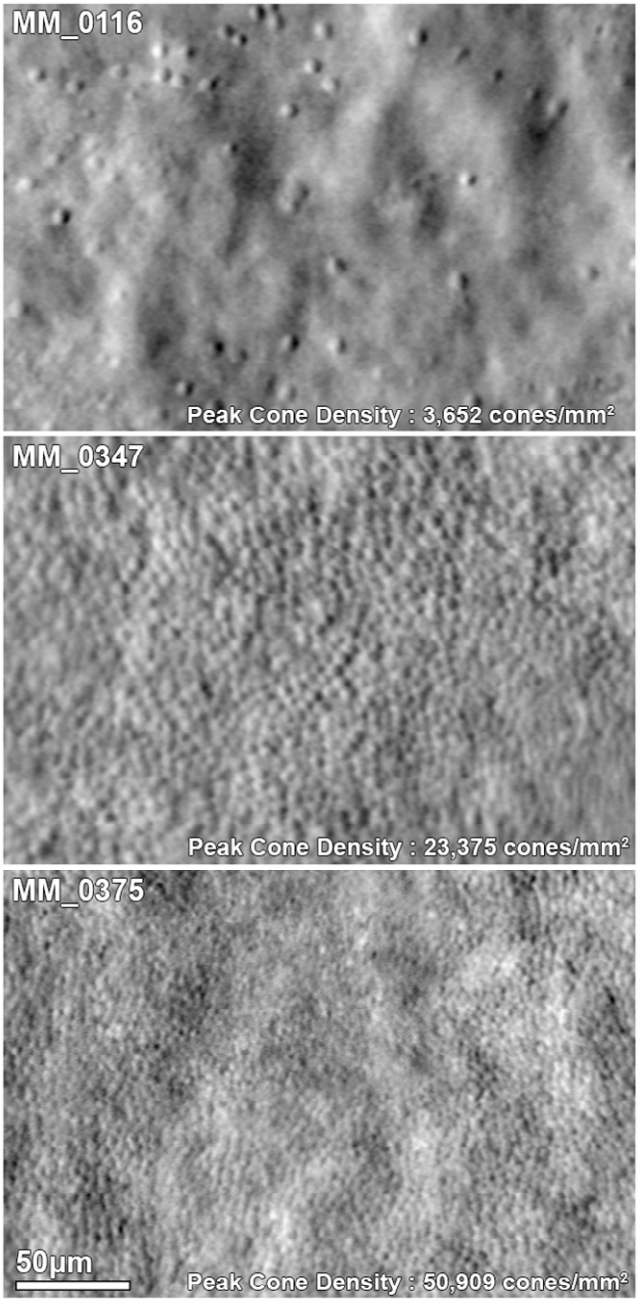


Adaptive Optics Imaging in a subject with *CNGB3*-ACHM

Inferior strips with the foveal centre at the top of the strip

(A) Confocal AOSLO montage. (B) Split-detection AOSLO montage

(C) Pseudocolour map, in red are represented the cone inner segments as imaged with split-detection filling the respective “dark” spaces in confocal image. The yellow/green colour in the picture is the result of the one-to-one matching of the photoreceptors in the two modalities (rods)



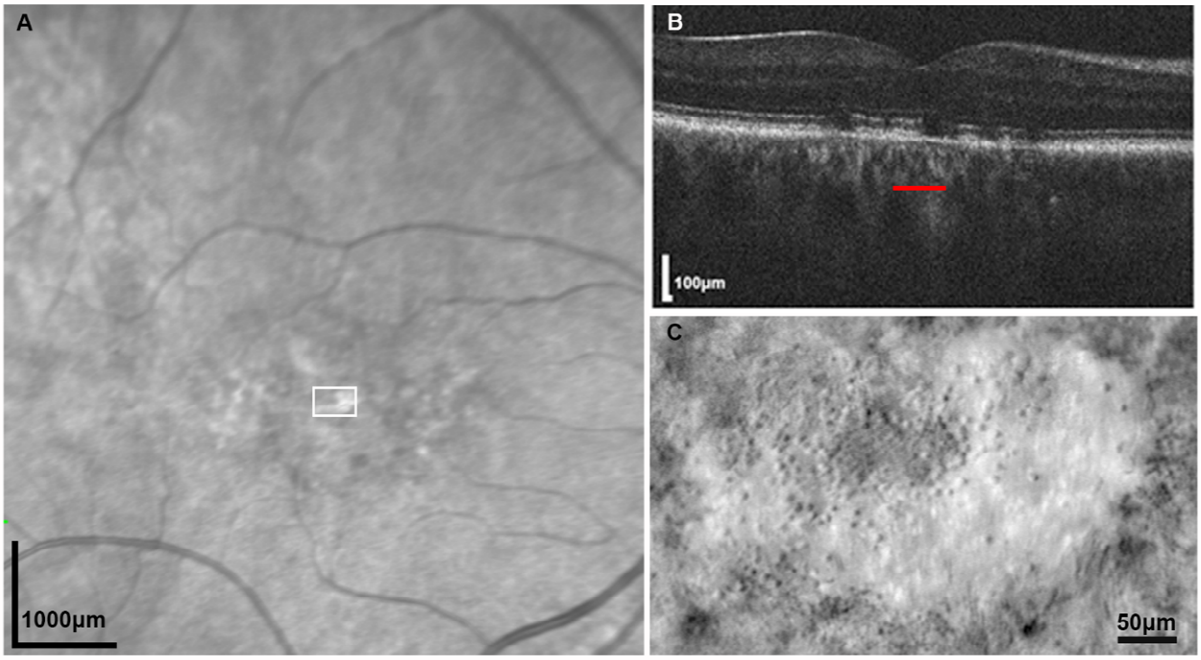
Variability in *CNGB3*-associated ACHM

Split-detection AOSLO images depicting the foveal centre. The foveal cone inner segments are visible with substantial variability between the three subjects.

Subject **MM_0116** with a sparse mosaic and the lowest peak foveal density of the cohort.

Subject **MM_0347** with a continuous mosaic and a peak foveal density closed to the average for the cohort.

Subject **MM_0375** with a continuous mosaic and one of the higher peak foveal density recorded.



Multimodal Imaging of *CNGB3*-associated ACHM

(A) Infra-Red imaging; the white box marks the exact area imaged with AOSLO split-detection at (C). The white square delineates an area of atrophy, visible also on horizontal OCT (B). The red bar on (B) represents the width of the white box on (A) and the exact width of AO image at (C). The line scan was taken in the middle of the lesion. Despite the atrophy, an "island" of remnant cone inner segments is visible on AOSLO imaging (C). The peak foveal density for that subject (MM_0123) was in the centre of that island at a value of 18992 cones/mm^2.