Title: Achromatopsia: clinical features, molecular genetics, animal models and therapeutic options

Running head: Achromatopsia

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ABSTRACT

Achromatopsia is an autosomal recessive condition, characterised by reduced visual acuity, impaired colour vision, photophobia and nystagmus. The symptoms can be profoundly disabling and there is no cure currently available. However, the recent development of gene-based interventions may lead to improved outcomes in the future. This article aims to provide a comprehensive review of the clinical features of the condition, its genetic basis and the underlying pathogenesis. We also explore the insights derived from animal models, including the implications for gene supplementation approaches. Finally, we discuss current human gene therapy trials.

Key words: achromatopsia, clinical trials, cone, cone dysfunction syndrome, gene therapy

INTRODUCTION

Achromatopsia (ACHM) is a cone dysfunction disease with an autosomal recessive mode of inheritance, affecting approximately one in 30,000 live births worldwide [1, 2]. A higher proportion of consanguineous marriage than that in the general population has been reported in ACHM [3]. The prevalence of the condition is particularly high in the small atoll of Pingelap in the Western Pacific, where approximately 6-10% of the population are affected, with around 30% of inhabitants being carriers [4]. ACHM is a predominantly stationary disorder characterised by lack of function of all three classes of cone photoreceptor from birth/early infancy. The nature and early onset of sight impairment can be severely disabling, with significant impact on activities of daily living [5]. Mutations in six genes are currently thought to be responsible for over 90% of cases of ACHM: *CNGA3, CNGB3, GNAT2, PDE6H, PDE6C* and *ATF6 [6]*. At present, there are no curative treatments for this condition, with current management being supportive to help minimise the effects of symptoms and optimise residual visual function. A great deal of progress has been made over the last decade that has increased the likelihood of novel treatments, including advances in the detailed phenotyping and genotyping of patients with ACHM, development of animal models and therapeutic tools, and the establishment of phase I/II human clinical trials assessing gene therapy. The purpose of this review is to summarise the latest developments in our understanding and characterisation of this condition, and to explore avenues of intervention that may become available in the near future.

CLINICAL FEATURES

ACHM is both phenotypically and genetically heterogeneous, with affected individuals displaying variability in the severity of their symptoms [7]. Affected individuals have marked

photophobia and pendular nystagmus of high frequency and low amplitude from birth or early infancy, associated with reduced visual acuity, impaired or absent colour vision, and central scotomata [8]. ACHM can be defined as 'complete' or 'incomplete', depending on the extent of cone photoreceptor dysfunction and hence severity of symptoms [2]. Individuals with complete ACHM have little or no cone function, visual acuity (VA) of 6/60 or less, and total absence of colour perception. A unique clinical feature of complete ACHM is paradoxical pupillary constriction to darkness, a feature known as the 'Flynn phenomenon', whereby patients display pupillary constriction on transfer to darkness after two to three minutes of exposure to bright light [4]. Those with incomplete ACHM have residual cone function, and therefore display a milder phenotype with VA between 6/24 and 6/36, and residual colour discrimination. Incomplete achromats may or may not display nystagmus and photophobia, and where present, these symptoms tend to be milder than in complete achromats. People with ACHM frequently have hypermetropic refractive errors [2], and less commonly, myopia. Visual acuity tends to be stable (hence the condition is considered functionally non-progressive), but nystagmus and photophobia may improve over time.

On electroretinography (ERG), cone-mediated responses are generally non-recordable or markedly reduced in ACHM, with normal or near-normal rod responses [9, 10]. However, defects in rod photoreceptor function and post-receptor responses have also been reported [11, 12]; for which there are several possible explanations including being secondary to a reduced number of rods, shorter rod outer segments, and potentially altered rod circuitry [13, 14].

On clinical examination, the fundus usually appears normal. However, some affected individuals may show subtle abnormalities at the macula, such as central pigment mottling, and/or loss of the foveal reflex. Rarely, atrophy of the retinal pigment epithelium (RPE) may

be evident at the fovea [15]. Detailed retinal imaging with optical coherence tomography (OCT) has demonstrated that patients with ACHM have variable outer retinal structure at the central macula including at the level of the photoreceptor inner segment ellipsoid layer (ISe) and RPE [16-18]. Sundaram et al. identified 5 distinct morphological appearances on spectral domain OCT (SD-OCT): (1) continuous inner segment ellipsoid (ISe), (2) ISe disruption, (3) ISe absence, (4) presence of a hyporeflective zone, and (5) outer retinal atrophy including RPE loss [7] (Fig. 1). The variable SD-OCT appearances did not correlate with age or genotype, with approximately 50% of patients having foveal hypoplasia [7].

Fundus autofluorescence (FAF) patterns show some correlation with OCT findings. FAF imaging reveals the distribution of lipofuscin across the posterior pole, and may be considered a surrogate marker of photoreceptor integrity [19]. Decreased FAF is suggestive of RPE atrophy and/or photoreceptor cell loss [20]. Increased FAF may signify an increased metabolic load prior to the onset of cell death [21]. FAF in patients with ACHM can be normal [22, 23], or demonstrate hyper- or hypo- autofluorescence [22] (Fig. 2). Greenberg et al. found that patients with RPE disruption on SD-OCT showed a corresponding area of reduced or absent FAF. Patients with ISe line disruption but no RPE damage also showed hypo-autofluorescence, localised to areas of photoreceptor loss and less marked than in those with RPE atrophy. Hyper-autofluorescence was generally noted in cases where the ISe line was preserved, and the authors proposed that this preceded photoreceptor cell loss [24]. Reports of age-dependent changes in FAF have suggested that ACHM can be progressive, with Fahim et al. proposing increased foveal autofluorescence in younger patients, and reduced autofluorescence with demarcated borders corresponding to outer retinal defects seen on SD-OCT in older patients [25].

Whilst some of the SD-OCT phenotypes may be associated with genotype (e.g. greater preponderance and degree of foveal hypoplasia in *ATF6*-associated ACHM), age has also been proposed as a factor [26], suggesting that ACHM may be a gradually degenerative condition. Thiadens et al. investigated 40 achromats cross-sectionally with SD-OCT, and proposed that cone cell decay began in early childhood, with retinal thinning correlating with age [16]. In addition, Thomas et al. reported that the presence of a hyporeflective zone and outer nuclear layer thinning were both age-dependent in a study of eight achromats [17]. However, whether FAF and/or SD-OCT appearances change with age remains the subject of debate, with many earlier studies being cross-sectional in nature and/or of relatively small size. Aboshiha et al. therefore evaluated 40 ACHM patients with serial SD-OCT and FAF, resulting in a longitudinal study of a large cohort (range of follow-up: 13-24 months) [22]. They identified three FAF patterns at baseline: (1) normal FAF pattern, (2) abnormal central increase in FAF, and (3) well-demarcated abnormal central reduction in FAF (Fig. 2). No change in the type of FAF pattern was observed between baseline and follow-up assessments in any patients, and no association between age and FAF pattern was observed. A statistically significant correlation between more disordered SD-OCT structure and a more abnormal FAF pattern was found. However, no correlation was found between age and SD-OCT findings. The authors concluded that whilst ACHM may be progressive in a minority of patients, the changes are likely to be slow and subtle, and not entirely dependent on age or genotype. There remains the need for more longitudinal studies with longer follow-up to probe this controversial area further.

Longitudinal assessments using more detailed retinal imaging techniques such as adaptive optics scanning light ophthalmoscopy (AOSLO) may also better demonstrate the earliest indicators of any structural progression in ACHM. AOSLO allows visualisation of

individual rod and cone cells *in vivo* [27, 28], and has confirmed the presence of cones in all patients with ACHM; albeit reduced in number compared to normal, with a wide range of cone densities (Fig. 3), and often a disconnect SD-OCT appearance [29]. Detection of photoreceptors with confocal AOSLO is dependent on relatively intact outer segment morphology to waveguide, and using this technique, reduced reflectance of residual cones and the presence of dark spaces (Fig. 3 (1A)) within the photoreceptor mosaic has been seen in ACHM [30, 31]. It is unknown whether these dark areas were devoid of cones, or whether they represented non-waveguiding cones. Following on from this, split-detector (non-confocal) AOSLO provided a transformational method to visualize cone inner segment structure independent of the integrity of the outer segment [32]. Using split-detector AOSLO in ACHM, it has now been shown that cone inner segments occupy the aforementioned dark areas seen in confocal AOSLO (Fig. 3 (2B/3B/4B/5B)), with significant implications on patient selection and gene therapy trials [32]. Split-detector AOSLO has also identified significant populations of photoreceptors in areas of no, low, or ambiguous ellipsoid zone reflectivity with *en face* OCT [29]. AOSLO studies of achromats have thus supported the fact that cones are still present, albeit in reduced numbers and in disrupted mosaics (Fig. 3). The cone mosaic is highly variable between patients [33], and demonstrates the need to assess individual patients when selecting those who may be most likely to benefit from interventional therapies. No significant differences in the cone mosaic have been observed between *CNGA3* and *CNGB3* ACHM (Fig. 3 (2-4)), with evidence suggesting that *GNAT2* ACHM (Fig. 3 (1B)) may be associated with a relatively more intact cone mosaic [31]. With regards to assessing progression of ACHM, Langlo et al. have demonstrated that foveal cone structure showed little or no change in their cohort of patients with *CNGB3*-associated ACHM over a follow-up

period of 6-26 months [34]. However, longer-term follow-up is needed in order to draw more definitive conclusions about progression over time.

PATHOPHYSIOLOGY AND MOLECULAR GENETICS

Five genes involved in the cone-specific phototransduction cascade have been implicated in ACHM. These are: *CNGA3* and *CNGB3* (encoding the α- and β-subunits respectively of the cyclic nucleotide-gated (CNG) cation channel 3 found in cone photoreceptor outer segments) [35, 36], *GNAT2* (encoding the catalytic α-subunit of the G-protein transducin) [37], and *PDE6C* and *PDE6H* (encoding the catalytic α- and inhibitory γ-subunits respectively of the cone photoreceptor-specific phosphodiesterase) [38, 39]. Most recently, *ATF6* has been identified as a sixth gene associated with ACHM. This encodes a transmembrane transcription factor, ATF6, expressed in all cells. ATF6 has a role in endoplasmic reticulum (ER) homeostasis [40]. It is unclear as to why mutations in this gene specifically cause cone dysfunction, though it has been suggested that ER stress-induced damage during retinal development is involved [41].

Over 100 mutations in *CNGA3* and over 50 in *CNGB3* have been identified as disease-causing in ACHM [42]. These genes were the first to be associated with this condition, and together, account for approximately 70-80% of all cases of ACHM [1, 2]. *CNGB3* mutations constitute approximately 40-50% of cases worldwide, and are more common in Europe and the USA (constituting around 60% of cases). *CNGA3* mutations underlie around 30-40% of cases worldwide, and are more common in the Middle East and China, making up around 60% of all cases in these regions [43].

Normal cone CNG channels are tetramers made of two α and two β subunits. Most *CNGB3* disease-causing mutations are nonsense, frame-shift, splice-site and large copy

number variations (CNVs) [44]. The most common *CNGB3*-related disease-causing mutation is a single base pair deletion, c.1148delC (p.Thr383IlefsTer13), which results in a frame-shift. This variant is found in over 70% of disease-causing alleles in *CNGB3* [45], and in keeping with other *CNGB3* variants, is predicted to result in complete lack of normal protein product and thereby absent cone function; thereby complete ACHM. However, Thiadens et al. have presented data suggesting that not all individuals with this mutation have complete ACHM [15]. The reason for this is poorly understood. In direct contrast, the vast majority of disease-causing *CNGA3* variants are missense in nature, wherein the end protein product, though present, is structurally abnormal. It has been suggested that some mutant CNGA3 proteins fail to be released from the ER with deleterious functional consequences [46, 47]. Complete lack or dysfunction of a protein product would be expected to account for cases of complete ACHM. In support of this theory, studies have shown that loss-of-function mutations in *CNGA3* lead to non-functional cone CNG channels, as CNGB3 subunits cannot form functional channels alone [48] [49]. However, in contrast, *in vitro* studies have shown that CNGA3 subunits in isolation can form functional ion channels [50]. Certain *CNGA3* mutations have been described in incomplete ACHM, suggesting that they allow residual function of the ion channel [51].

GNAT2 was the third gene found to be associated with ACHM, and accounts for less than 2% of all cases [42]. *GNAT2* mutations have been found in cases of both complete and incomplete ACHM [23, 52]. The variant c.461+24G>A which results in abnormal splicing *in vitro* is thought to result in an incomplete ACHM phenotype [52]. Other mutations in this gene have been found to produce a truncated non-functional transducin protein [53]. *PDE6C* mutations also account for less than 2% of ACHM cases, and *in vitro* studies have demonstrated that missense mutations result in enzymatic dysfunction, ranging from

reduced to complete loss of activity [54]. *PDE6H* mutations are the rarest cause of ACHM, accounting for less than 1% of affected individuals. The most recent gene, *ATF6*, accounts for 1-2% of ACHM with sequence variants found to cause both complete and incomplete ACHM [40].

There appears to be no definite correlation between genotype and phenotype in ACHM [2, 15]. However, some interesting potential relationships have been observed. Imaging studies have suggested that patients with *GNAT2*-ACHM have relatively milder outer retinal disruption and better-preserved cone mosaics compared to those with *CNGA3*- or *CNGB3*-ACHM, as evidenced by SD-OCT and AOSLO respectively [31]. There is evidence suggesting that foveal hypoplasia is more frequent and marked in *ATF6*-ACHM compared to ACHM associated with phototransduction dysfunction [40], leading to the suggestion that *ATF6* has a crucial role in foveal development.

Whilst ACHM is relatively well-characterised molecularly, missing mutations may be identified in future to account for the remaining minority of cases [44, 55].

ANIMAL MODELS OF DISEASE

Animal models of disease have provided significant insight into the pathogenesis of ACHM. Small (murine) and large (canine and ovine) animal models exist for the major genetic variants of ACHM. The *CNGA3* knockout mouse, with a homozygous deletion in an exon encoding functional portions of the CNGA3 protein, has an almost total lack of cone function, with a reduced total number of cones, and structural abnormalities in residual cones. Loss of cone function correlates with selective cone degeneration [56]. Furthermore, loss of CNGA3 has been shown to impair the targeting of cone opsins to cone outer segments, and to downregulate the expression of other proteins involved in the phototransduction cascade,

with subsequent apoptosis of cones [57]. A naturally-occurring animal model for *CNGA3*-ACHM is the Awassi sheep. Shamir et al. [58] described congenital visual impairment in lambs of this breed, with reduced day vision and normal night vision. Electrophysiological studies of affected four month-old lambs revealed markedly reduced/absent cone function, despite the presence of cones histopathologically [59]. Affected lambs carry homozygous missense mutations in *CNGA3* [60]. Naturally-occurring canine models of *CNGA3*-ACHM have also been described, homozygous for either a missense mutation (p.R424W) or a deletion (p.V644del) in *CNGA3*. The p.R424W variant results in complete loss of cone function *in vivo*, and absent CNG channel activity *in vitro*. The p.V644del mutation resulted in failure of normal CNGA3 subunit assembly *in vitro* [61].

Naturally-occurring canine models for human *CNGB3*-ACHM have also been identified - the Alaskan malamute and German short-haired pointer [62]. Electrophysiological assessment revealed progressive loss of cone function over time, ranging from reduced in pups to absent function in adulthood, associated with progressive cone degeneration [63].

A naturally-occurring mouse model for *GNAT2*-ACHM has been described [64]. Cone photoreceptor function loss 3 *(cpfl3)* mice, harbouring a homozygous missense mutation, showed reduced cone-mediated ERG responses at three weeks of age, with complete loss at nine months. Another naturally-occurring mouse model with a missense variant has absent cone ERG responses [65]. Progressive loss of cones and increasing structural abnormalities over time have been shown, associated with evidence of M-opsin mislocalization.

A murine model for *PDE6C*-ACHM, the cone photoreceptor function loss 1 *(cpfl1)* mouse, has absent cone ERG responses at three weeks of age [54]. Rapid, progressive loss of cones has been described, such that only limited numbers of cones are evident at the age of five months.

Interestingly, in contrast to the aforementioned models, neither the *PDE6H*- nor *ATF6*-knock-out mouse models have been shown to mimic human ACHM [40, 66], and this may be due to differences between species in the vulnerability of the visual signal transduction system to the effects of these variants.

CURRENT MANAGEMENT AND PRE-CLINICAL THERAPEUTIC APPROACHES

At present, there is no cure for ACHM, with clinical management relating to genetic counselling, the provision of low vision aids to optimise vision, and the use of tinted contact lenses and spectacles to alleviate photophobia [2]. However, despite the use of the best available equipment and the input of specialist services, everyday tasks often continue to pose significant challenges to these patients, with implications on their social, personal and professional lives [5]. Thus, there has been much work aiming to identify a definitive treatment that will improve their quality of life. Since ACHM results from autosomal recessive loss-of-function mutations, one of the main therapeutic avenues that has been explored is that of gene augmentation therapy, whereby affected cone cells are supplied with healthy copies of the mutant gene. The availability of animal models of disease and recombinant adeno-associated viral vectors (rAAVs) to safely transfer retinal genes has fuelled research in this area. Promising results for gene supplementation have been shown in animal models of *CNGA3*-, *CNGB3*- and *GNAT2*-associated ACHM.

Gene supplementation in a knock-out *CNGA3* mouse model has shown restoration of cone-specific visual processing in the central nervous system, where cone cells have lacked function since birth [67]. Gene therapy also had beneficial effects on retinal morphology, delayed cone cell death and reduced the inflammatory response of Müller glia cells. Moreover, improved cone-mediated vision-guided behaviour was demonstrated [67].

Subsequent studies suggested the effects lasted for at least eight months, and that rescue was observed in older animals at one to three months of age [43]. Further data published on the efficacy and durability of gene supplementation in this model demonstrated a therapeutic effect in mice treated at two weeks of age, as well as those treated at three months old, sustained throughout the observation period of 12 months; although the average ERG amplitudes differed, suggesting that age at treatment (and thus potentially the extent of photoreceptor degeneration) may affect outcome [68]. Successful rescue has also been shown in a naturally-occurring *CNGA3* mouse model, where treatment resulted in restoration of cone ERG responses, improvement of visual acuity and contrast sensitivity, and halted cone degeneration [69]. Promising results have been replicated in the Awassi sheep model of *CNGA3*-ACHM, where gene therapy led to an improvement in the cone ERG and daylight vision, with effects maintained for up to three years post-treatment [70]. A more recent study has similarly shown rescue with gene therapy in the Awassi model [71]. The success of gene therapy in this case is of particular significance, since it occurred in a large animal model with a genotype in keeping with majority of *CNGA3*-ACHM genotypes (missense variants) rather than knock-out/premature termination models.

Successful gene supplementation has also been described in models of *CNGB3*-associated disease, with improvement of cone function and daylight vision for at least 33 months in two canine models [72]. Older animals, particularly those over one year old, with more advanced disease showed less improvement. To explore this further, gene replacement has also been undertaken *after* intravitreal ciliary neurotrophic factor (CNTF) treatment. CNTF causes transient dedifferentiation of photoreceptors, a process during which the cells become morphologically immature, with resulting decreased function and gene expression. With adjunctive CNTF treatment, gene supplementation restored cone function in all mutant

dogs treated at the ages of 14 to 42 months [73]. Interestingly, restoration of cone function (as evidenced by restoration of cone ERG amplitudes of up to 90% of normal), has been observed in *CNGB3* knock-out mice even when treated at a relatively advanced stage of disease (six months old) [74]. However, normalisation of visual acuity was only noted in mice treated at an earlier stage (two to four weeks of age).

Encouraging results have also been obtained in an animal model of *GNAT2*-ACHM, where gene supplementation was observed to improve cone-mediated ERGs and optomotor behaviour in mice; with effects maintained for at least seven months [75].

Delivery of genes via the subretinal route, although shown to transfect photoreceptors most efficiently, may be associated with retinal damage caused by injection-induced retinal detachment, particularly when the subretinal vector bleb involves the fovea. Du et al. studied an alternative route of viral vector administration, using the *CNGA3(-/-)/Nrl(-/-)* mouse, a cone-dominant model with *CNGA3* channel deficiency, which partially mimics the foveal architecture of human *CNGA3*-ACHM [76]. Using intravitreal delivery, they demonstrated improved cone structure and function, as indicated by restoration of the cone-mediated ERG, optomotor responses and cone opsin immunohistochemistry. This encourages further exploration in larger animal models that have retinal structure more similar to the human.

CLINICAL TRIALS

Promising results from gene therapy in animal models of ACHM have given hope for similar interventions in patients, and the successful use of human promotors and transgenes in mouse models has reinforced this possibility [69, 72, 74, 75].

ACHM has several features that suggest it may be particularly amenable to gene therapy in humans. Firstly, effective improvement in cone function would provide a clear, rapid and reliable measure of outcome. Although ACHM may be associated with progressive cone degeneration, the rate of degeneration is considered to be slow, and thus the extended survival of cones, in whom function can potentially be rescued, provides a wide window of opportunity during which gene supplementation could potentially improve cone-mediated vision. Preclinical data suggests that intervention at a younger age may lead to better outcomes. This may in part be due to better preserved cones at this age, but moreover also due to greater visual plasticity. However, it is anticipated that gene supplementation would offer some benefit across a range of treatment ages.

Gene therapy in ACHM depends on the presence of cones in which function can potentially be restored, i.e. those that are not significantly degenerate. Thus, there has been a drive to obtain natural history data in ACHM patients, to ascertain features which identify the patients most likely to benefit from gene therapy, as well as define the optimal window for intervention (described in previous *Clinical Features* section).

Several clinical trials in patients with inherited retinal diseases have shown that subretinal vector administration has led to visual improvement without significant safety concerns [77-80]. This observation, in conjunction with favourable results from gene therapy in animal models of ACHM, has led to the initiation of translational studies investigating gene supplementation in the more common forms of ACHM, i.e. *CNGA3*- and *CNGB3*-ACHM. A phase I/II study commenced in November 2015, as a collaboration between the University Hospital Tuebingen and Ludwig-Maximilians - University of Munich aimed to assess the safety and efficacy of subretinal injection of rAAV.hCNGA3 in patients with *CNGA3*-ACHM, using a

dose-escalation protocol (NCT02610582). The interventional phase was complete by the end of November 2016, and study results are anticipated. In addition, there is a further phase I/II trial in the USA aiming to assess the safety and efficacy of a recombinant AAV vector expressing *CNGA3* (NCT02935517); the trial is open but has not to date started recruitment (December 2017). Other active gene therapy clinical trials include a UK-based open label, multi-centre, phase I/II dose escalation trial of the safety and efficacy of subretinal AAV2/8-hCARp.hCNGB3 in adults and children with *CNGB3*-ACHM, commenced in January 2017 (NCT03001310). There is also a trial based in the US, commenced in February 2016; an open-label, phase I/II study of the safety and efficacy of subretinal rAAV2tYF-PR1.7-hCNGB3 in *CNGB3*-ACHM (NCT02599922). Results for all these ongoing trials are eagerly awaited by both clinicians and patients, given the implications that positive results may have on the future management of ACHM.

Whilst gene therapy holds great hope for the treatment of ACHM in the future, there are caveats to be considered. Firstly, the individuals selected need to have a population of 'rescuable' cones, and SD-OCT and AOSLO will help identify patients suitable with regard to this aspect (as discussed previously in *Clinical Features*). Study subjects should also be able to perform assessments of retinal function reliably and reproducibly, such that pre- and post-intervention data can be accurately compared and useful inferences made about the effect of therapy. Patient factors such as nystagmus, poor fixation and photophobia may hinder their performance in certain tests, and therefore, may limit the quality of data that can be collected from some achromats. Thus, careful selection of potential subjects is essential if gene therapy trials are to provide reliable results about the effects of treatment.

CONCLUSIONS

ACHM is a significantly disabling condition from birth/early infancy, with patients encountering profound difficulties in everyday activities. Despite the provision of aids and assistance from specialist services, the management of ACHM remains suboptimal. There is therefore a need to identify a definitive therapy, in order to alleviate the symptoms that patients experience. Based on preclinical data, there is cautious optimism for the potential role of gene therapy in improving cone function in affected humans, with possible benefits including improved visual acuity, improved colour perception, and reduction of photophobia. Advances in genetic molecular techniques to accurately genotype patients, as well as in imaging and functional investigations to phenotype patients in greater detail than previously possible, will aid in identifying affected individuals with the potential to benefit from gene supplementation, and also in creating protocols for monitoring the effects of intervention. The forthcoming results of gene therapy trials currently in progress may revolutionise the future of ACHM management.

DECLARATION OF INTEREST

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FIGURE LEGENDS

Figure 1. Representative images of the five spectral domain optical coherence tomography phenotypes in achromatopsia. (A) continuous inner segment ellipsoid (ISe), (B) ISe disruption, (C) ISe absence, (D) presence of a hyporeflective zone, and (E) outer retinal atrophy including RPE loss. Scale bar: 200µm.

Figure 2. Representative images of the three different fundus autofluorescence (FAF) phenotypes in achromatopsia. (A) reduced FAF signal centrally with a well-demarcated border, (B) normal FAF appearance, and (C) a central increase in FAF.

Figure 3. Adaptive optics scanning light ophthalmoscopy (AOSLO) imaging in achromatopsia (ACHM) (1A) Confocal AOSLO (cAOSLO) of the cone mosaic 2^{o} away from the fovea in a subject with *CNGB3*-ACHM. White arrows highlight some of the dark spaces within the photoreceptor mosaic. (1B) cAOSLO of the cone mosaic in a subject with *GNAT2*-ACHM, at the same eccentricity and scale. The photoreceptor mosaic appears continuous and regular compared to (1A). (2-5) Left column shows cAOSLO (A) and right column shows split detection (SD) AOSLO (B). All images depict the foveal centre with cAOSLO and SD-AOSLO co-localised. (2A/2B and 3A/3B) and (4A/4B and 5A/5B) show subjects with *CNGA3*- and *CNGB3*-ACHM respectively. In cAOSLO, cones appear dark and the mosaic appears irregular, due to loss of cone waveguiding properties. In contrast, on the corresponding SD-AOSLO images, the foveal cones' inner segments are visible with substantial variability between the two subjects.

All images were acquired using a custom-built AOSLO housed at University College London/Moorfields Eye Hospital, London, UK. Dimensions: 200µm × 100µm, Scale bar: 40µm.