Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Behavioral Sciences

# Single-cone imaging in inherited and acquired colour vision deficiencies

Rigmor C Baraas, Hilde R Pedersen and Lene A Hagen

Colour vision deficiencies are common in humans and occur both as a consequence of inherited cone opsin mutations, altering the number or function of the different cone types expressed in the retina, and acquired through secondary disruption of cone function and structure. This review describes recent advances made in understanding colour vision deficiencies from combining knowledge about cone opsin genes with single-cone imaging in living humans. Examination of the effect of the opsin gene mutations upon the cone mosaic and colour vision phenotypes shows that not all inherited colour vision deficiencies are stationary and some inherited congenital eye diseases may cause impaired colour vision as a consequence of arrested development.

**Address**
National Centre for Optics, Vision and Eye Care, Faculty of Health and Social Sciences, University of South-Eastern Norway, Hasbergsvei 36, 3616 Kongsberg, Norway

Corresponding author: Baraas, Rigmor C (rigmor.baraas@usn.no)

**Current Opinion in Behavioral Sciences** 2019, **30**:55–59

This review comes from a themed issue on **Visual perception**

Edited by **Hannah Smithson** and **John S Werner**

https://doi.org/10.1016/j.cobeha.2019.05.006



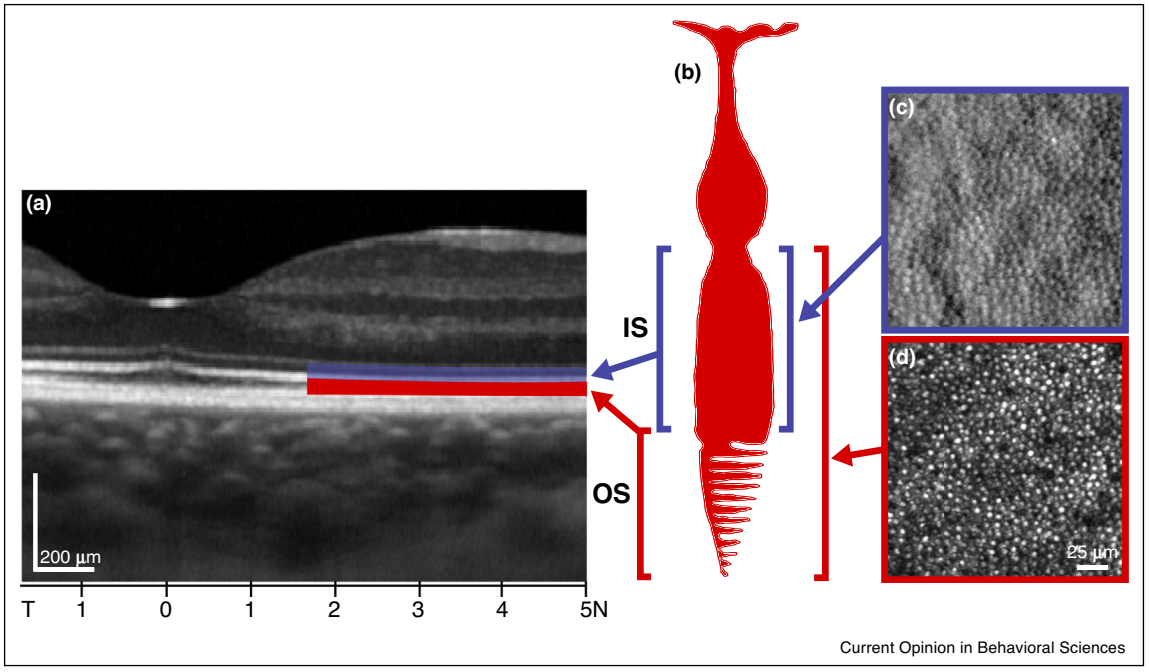
## Introduction

Direct visualisation of single photoreceptor cells in eyes of living humans was achieved for the first time in 1999 [1], paving the way for a broader and deeper understanding of the cone mosaic and colour vision. *In-vivo* microscopic imaging of the retina was made possible through adding adaptive optics (AO) — a wavefront sensor to measure the ocular monochromatic aberrations and a deformable mirror to correct for the aberrations — in ophthalmoscopic imaging systems. Several systems have been developed over the years, but it is the multimodal version of AO scanning light ophthalmoscope (AOSLO), incorporating both confocal and non-confocal detection that has helped us make the most important discoveries [2,3]. Such a system allows for simultaneous imaging of both the confocal waveguided light from the outer segments and non-confocal backscattered light from the retina, revealing the structure of the inner segments of the cones (in direct temporal and spatial correspondence as illustrated in Figure 1). This is of significance, because remnant inner segments can now be observed in conditions where cone opsin mutations render the outer segments dysfunctional or non-functional so that it cannot waveguide light properly. Single-cone imaging in living human eyes has allowed for major advancements in understanding the properties of the cone mosaic, the distribution and organisation of different cone types [4], structural and functional changes as a consequence of disruptions caused by genetic mutations or disease. It has become a tool that allows for tracking longitudinal changes of the cone mosaic, including the effect of medication and genetic treatment [5••]. Examination of the effect of the opsin gene mutations upon the cone mosaic and colour vision phenotypes shows that not all inherited colour vision deficiencies are stationary or benign [6–8,9••] and some inherited eye diseases may cause impaired colour vision as a consequence of arrested development [10•,11••]. In this paper, we discuss recent advancements in understanding the links between retinal structure, function and colour perception, elucidating associated consequences of colour vision loss, either as a result of opsin gene mutations or secondary to disease or genetic mutations that disrupt retinal development.

## Studying the variation in cone photoreceptor mosaic in living humans with impaired colour vision

There are three lines of work in the study of variations in the cone photoreceptor mosaic and colour vision deficiencies that we will focus on. Firstly, we will review the differences between cone opsin mutations that give rise to inherited colour vision deficiencies (impaired colour discrimination, but not colour blindness) and their effect on retinal structure and natural history (stationary or progressive). The aim here is to understand why some inherited red–green (protan/deutan) and blue–yellow (tritan) colour vision deficiencies may be progressive. We will then briefly touch upon variation in cone photoreceptor mosaic in inherited cone dysfunctions that give rise to colour blindness (achromatopsia), and the importance of individual assessment when considering who will possibly benefit from genetic treatment to restore cone function. Finally, we will mention the effect of other genetic mutations that arrest the development of the central parts of the retina, and the structure–perception correlations between single-cone imaging, optical

**Figure 1**




Single-cone imaging of living humans. **(a)** OCT image showing the layered structure of the central retina from 1.5° temporally (T) to 5° nasally (N) with the characteristic foveal pit (centred at 0°) of a person with normal spatial and colour vision. Scale bar is 200 μm. The layers including the inner (blue) and outer (red) segment structures are outlined. **(b)** A sketch of a cone photoreceptor with its inner and outer segments. A cone photoreceptor is about 50 μm long, and its inner segment is about 1 μm in diameter in the fovea and about 2 μm at 5° eccentricity in a healthy adult retina. AOSLO images of non-confocal backscattered light from the retina, revealing the structure of **(c)** the inner segments and the confocal waveguided light from **(d)** the outer segments of the cones. Scale bar is 25 μm.

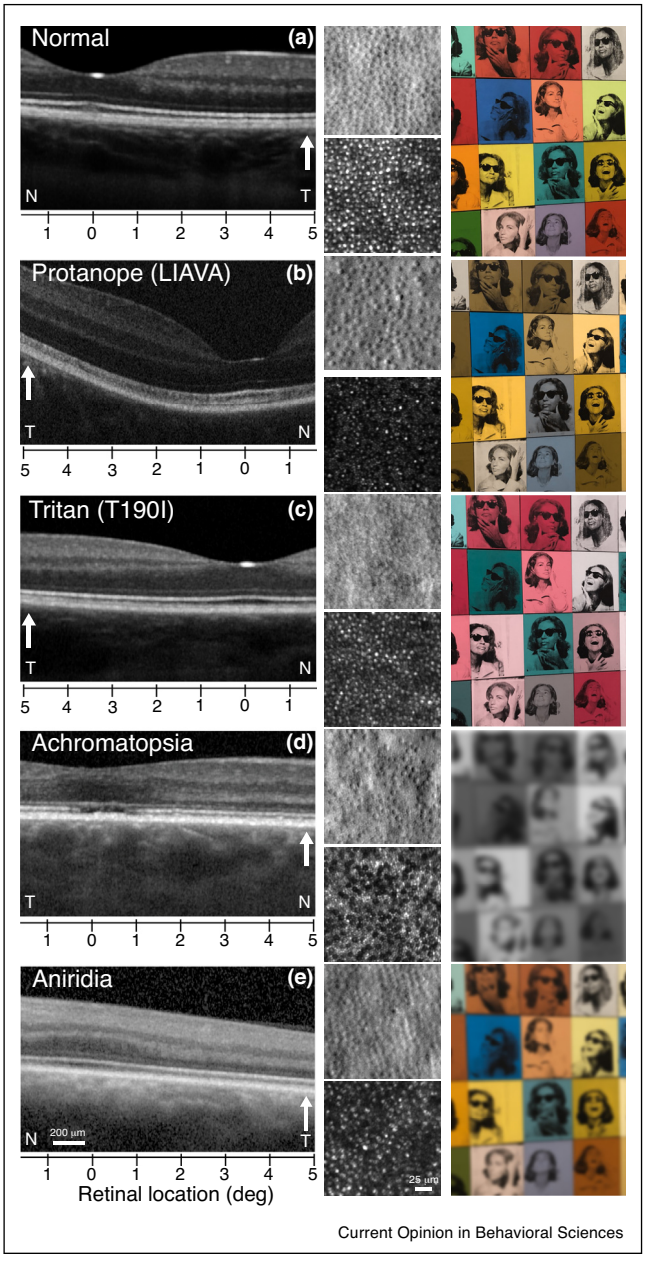
coherence tomography (OCT) imaging and colour vision impairment.

## The cone mosaic in inherited red–green colour vision deficiencies

Inherited colour vision deficiencies are a consequence of cone opsin mutations. Mutations in the genes for the human long-wavelength (L) or the middle-wavelength (M) cone opsin, localised on the X-chromosome at Xq28 (genetic designation OPN1LW and OPN1MW), give rise to red–green colour vision deficiencies. Mutations in the genes for the human short-wavelength (S) cone opsin, localised to an autosome on chromosome 7 at 7q32 (genetic designation OPN1SW), give rise to blue–yellow colour vision deficiencies [12]. There is a large variation in the arrangement of cone opsin genes giving rise to large differences in the associated degree of colour vision deficiency. Two types of opsin mutations have been associated with red–green dichromatic colour vision; one encodes partial or complete deletion or replacement of photopigment of a different spectral class, the other encodes a photopigment that does not function normally (random missense mutation). Recently, several lines of work have been carried out to elucidate how these mutations might be expressed and what effect they might have on the cone mosaic. Carroll *et al.* used AO flood-illuminated imaging to examine the cone mosaic in red–green dichromatic individuals [7,8]. The participants harboured either a mutation that encodes the replacement of L with M photopigment, but with a deleterious combination of nucleotides at normal polymorphic positions (LIAVA, often referred to as an L/M interchange mutation) [12], or the mutation C203R[1] in the M opsin gene, that encodes a non-functional photopigment [7,8]. They showed that the cone density associated with the LIAVA mutation was similar to that in normal trichromats, albeit with a mottled and less regular appearance. The C203R mutation was associated with lower cone density, but a regular cone mosaic interleaved with dark regions thought to have been degenerated cones expressing the non-functional photopigment. The LIAVA cones appeared to be still present, having perhaps slowly progressively lost function, whereas the C203R cones had completely degenerated early in foveal development. Further studies with confocal AOSLO imaging, including another random missense mutation (W177R),

[1] Missense mutation where the cysteine residue at amino acid position 203 of the pigment molecule is replaced with arginine.

**Figure 2**




Single-cone images of living humans with different degrees of spatial and colour vision. The left column shows OCT images revealing the layered structure of the central retina of persons with **(a)** normal colour vision and normal cone opsin genes, **(b)** red–green (protan) colour vision deficiency caused by an M interchange mutation (LIAVA, subject MM0142 [9••]), **(c)** blue–yellow (tritan) colour vision deficiency caused by an S opsin mutation (T190I, subject K1539 [16]), **(d)** achromatopsia (colour blind) and **(e)** aniridia (subject 5120 [11••]). Scale bar is 200 μm. The disruption in the layer with the outer segments is clearly visible in achromatopsia (d) as is the foveal hypoplasia (lack of foveal pit) in aniridia (e). The corresponding single-cone images of each participant are shown in the middle column, revealing the structure of the inner (top) and outer segments (bottom) of cone and rod photoreceptors at about 5° eccentricity (marked by the white arrows). Scale bar is 25 μm. The images show the differences in observed cone density when cone opsin mutations render the outer segments dysfunctional (b: LIAVA) or genetic mutations render the outer segments non-functional (d: achromatopsia) not waveguiding light properly. The images in the right column are alterations of a photograph taken by Baraas of *Ethel Scull 36 Times* (1963) by Andy Warhol to simulate the corresponding loss of spatial and colour vision.

deletion of exons and other L/M interchange mutations (LVAVA, LIAVS, LVVVA), have expanded on these conclusions [13,14]. L/M interchange mutations and exon 2 deletions were shown to be associated with more disruptive retinal structure changes in the fovea compared with random missense mutations. This included damage to neighbouring S cones and rods. Recently, multimodal AOSLO imaging of participants with X-linked cone opsin mutations, associated with red–green colour vision deficiency (both anomalous trichromacy and dichromacy) [15•], has shown that the dark regions observed in confocal images appears to be regions with reduced or lost cone function (poor or no waveguiding of light), because intact cone inner segments are clearly visible [9••] (see Figure 2b). Further to this, the large between-individual variation in retinal structure, function and colour perception appears to be associated with both the types of L/M opsin mutation and the ratio of expression of first versus downstream genes on the gene array [15•].

## The cone mosaic in inherited blue–yellow colour vision deficiencies

The types of opsin mutations associated with blue–yellow colour vision deficiency appear more similar to mutations in the gene encoding rhodopsin (the rod photoreceptor pigment) giving rise to retinitis pigmentosa [6,16], than to L and M opsin mutations, occurring at amino acid positions expected to result in photoreceptor degeneration [12]. Baraas *et al.* used AO flood-illuminated imaging including retinal densitometry to examine the cone mosaic in a family with an S opsin mutation [6]. The eldest member was diagnosed to be a dichromat lacking S cone function (tritanopia), whereas a younger member only had a mild degree of tritan colour vision deficiency [6]. Images of their photoreceptor mosaic showed that cone density associated with this specific mutation (R283Q) was within normal limits, but the eldest tritanopic member of the family lacked S cones and had a more irregular mosaic than observed in the younger family member and in normal controls. The R283Q cones appear to be progressively degenerating, with tritan colour vision deficiency only being manifest when a sufficient number of S cones have degenerated. A similar observation was made with another S opsin mutation (T190I) in that older family members were more severely affected than those who were younger [16]. Results from assessing colour vision function at different light levels, before and after bleaching and dark adaptation, corroborate the suggestion that S opsin mutations are more akin

to rhodopsin mutations, and that there is a progressive element. Figure 2c show images of the retinal structure in a participant with the T190I S opsin mutation. Cone density appears normal, but retinal densitometry is needed to ascertain whether some of the dark areas on the confocal image are poorly functional S cone outer segments. Not all inherited tritan colour vision deficiencies may behave in the same way; it will be down to the type of opsin mutation and what effect this has on the cone photoreceptor function at a given time in life. Longitudinal imaging with multimodal AOSLO, combined with retinal densitometry, are required to improve understanding to what degree cone inner segment structure is retained in S cone dysfunction.

The advancements brought forward by combining analyses of opsin genotype with colour vision and photoreceptor phenotypes from single-cone imaging in living humans have been imperative for understanding the potential for gene therapy and to restore cone function. Although some may deem this not to be important for those with a stationary and benign protan, deutan or tritan colour vision deficiency, there is a strong case for understanding the whole aspect from variations within normal colour vision and cone mosaics through life, and even more so for being able to offer genetic treatment to those who are de facto colour blind as a consequence of inherited cone dysfunction.

## The cone mosaic in the colour blind (achromats/monochromats)

Achromatopsia and blue cone monochromacy (complete and incomplete colour blindness) are typically stationary cone dysfunctions which give rise to impaired central visual acuity, nystagmus and photophobia. The cone mosaic, when imaged with AO flood systems [17] or confocal AOSLO [18], appears to have reduced reflectance and dark areas where residual cone structures may reside. With confocal and non-confocal detection AOSLO systems, it has been shown that there are intact cone inner segment structures [3] in these dark areas, even when cone function is markedly reduced or lost [19], but with considerable variation between participants [5••], see also Figure 2d. The degree of variation in the remains of cone structure in achromatopsia, within the same and between different mutations, underscores the importance of individual assessment when considering restoring cone function with gene therapy.

## The cone mosaic and colour vision associated with arrested foveal development

Genetic mutations associated with albinism and aniridia are associated with arrested development resulting in foveal hypoplasia. This underdevelopment of the central parts of the retina is typically associated with reduced visual acuity, nystagmus and photophobia. It has been shown that there is a correlation between cone density and the degree of foveal hypoplasia, assessed by spectral domain OCT images, in both albinism and aniridia [11••,20]. The degree of foveal hypoplasia correlates with the degree of red–green colour vision in aniridia [10•], those with poorest red–green sensitivity have the lowest cone density [11••], see Figure 2e. It is not known if this is also the case in albinism. It is assumed that differences in retinal ganglion cell density and/or cone-midget retinal ganglion cell pathways are factors that may contribute to variation in colour vision between individuals with the same grade of foveal hypoplasia.

## Conclusions

The utilisation of multimodal adaptive optics scanning light ophthalmoscopy to image single cone outer and inner segments has allowed us to answer some important questions about the effects different types of opsin mutations associated with inherited colour vision deficiencies and other inherited eye diseases have on cone structure and function (Figure 2). Because of developmental mechanisms, including epigenetic factors and experience-dependent change and individuation [21] that decide the number of cones any individual express on their retina, both cross-sectional and longitudinal studies utilizing multimodal single-cone imaging will continue to be important tools to gain an even better understanding for cellular retinal anatomy and colour perception. Additional advancements, such as functional measurements of single cones during imaging, are expected to transform our understanding of the correlations between the organisation of different cone types and colour vision in the future [22]. The importance of understanding these correlations is not only for the benefit of those with the disease or for treatment of those, but also for understanding what constitutes normal healthy development and who might be at risk for developing age-related disease. There is a large variation in the number of cones in the macular region of those with normal colour vision [23] and associated variation in colour vision loss with age [24]. This indicates that there is a real chance that those with low redundancy of cones are the ones who experience early (colour) vision loss, whereas those with high redundancy of macular cones may retain full visual function for longer. Only longitudinal studies tracking both colour vision and multimodal single-cone imaging in living humans with known opsin gene array will allow us to learn more.

## Conflict of interest statement

Nothing declared.

## Acknowledgements

The authors thank Stuart J Gilson, Elise Dees Krekling, Maureen Neitz and Alfredo Dubra for their contribution to this work. They also thank Joseph Carroll and Emily Patterson for sharing images of one of their participants with the LIAVA mutation.

The authors have received support from the Research Council of Norway, the Oslofjord Fund and Aniridia Norway for their research. The research

facilities at the National Centre for Optics, Vision and Eye Care were constructed and funded through a strategic commitment by the University of South-Eastern Norway in collaboration with the eye care industry in Norway.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest


1. Roorda A, Williams DR: **The arrangement of the three cone classes in the living human eye**. *Nature* 1999, **397**:520-522.

2. Dubra A, Sulai Y: **Reflective afocal broadband adaptive optics scanning ophthalmoscope**. *Biomed Opt Express* 2011, **2**:1757-1768.

3. Scoles D, Sulai YN, Langlo CS, Fishman GA, Curcio CA, Carroll J, Dubra A: **In vivo imaging of human cone photoreceptor inner segments**. *Invest Ophthalmol Vis Sci* 2014, **55**:4244-4251.

4. Hofer H, Carroll J, Neitz J, Neitz M, Williams DR: **Organization of the human trichromatic cone mosaic**. *J Neurosci* 2005, **25**:9669-9679.

5. •• Georgiou M, Litts KM, Kalitzeos A, Langlo CS, Kane T, Singh N, Kassilian M, Hirji N, Kumaran N, Dubra A, Carroll J *et al.*: **Adaptive optics retinal imaging in cnga3-associated achromatopsia: retinal characterization, interocular symmetry, and intrafamilial variability**. *Invest Ophthalmol Vis Sci* 2019, **60**:383-396.

Georgiou *et al.* used multimodal AOSLO retinal imaging to investigate the cone mosaic in participants with *CNGA3*-assosiated achromatopsia. Non-confocal AOSLO images revealed irregular and disrupted remnant cone mosaic with large variability among individuals, even for the same mutations. The paper shows the usefulness of multimodal AOSLO in understanding disease pathogenesis in achromatopsia. Large between-individual variability underlines the importance of assessing persons with ACHM on an individual basis in selection for clinical trials.

6. Baraas RC, Carroll J, Gunther KL, Chung M, Williams DR, Foster DH, Neitz M: **Adaptive optics retinal imaging reveals s-cone dystrophy in tritan color-vision deficiency**. *J Opt Soc Am A Opt Image Sci Vis* 2007, **24**:1438-1447.

7. Carroll J, Baraas RC, Wagner-Schuman M, Rha J, Siebe CA, Sloan C, Tait DM, Thompson S, Morgan JI, Neitz J *et al.*: **Cone photoreceptor mosaic disruption associated with cys203arg mutation in the m-cone opsin**. *Proc Natl Acad Sci U S A* 2009, **106**:20948-20953.

8. Carroll J, Neitz M, Hofer H, Neitz J, Williams DR: **Functional photoreceptor loss revealed with adaptive optics: an alternate cause of color blindness**. *Proc Natl Acad Sci U S A* 2004, **101**:8461-8466.

9. •• Patterson EJ, Kalitzeos A, Kasilian M, Gardner JC, Neitz J, Hardcastle AJ, Neitz M, Carroll J, Michaelides M: **Residual cone structure in patients with x-linked cone opsin mutations**. *Invest Ophthalmol Vis Sci* 2018, **59**:4238-4248.

Patterson *et al.* used multimodal AOSLO retinal imaging to assess residual cone structure in participants with *OPN1LW* or *OPN1MW* opsin mutations which introduce splicing defects leading to a lack of or reduced amount of functional photopigment in cones expressing the mutation. Confocal images revealed disrupted cone reflectivity and reduced cone density, whereas non-confocal images revealed residual cone inner segments could be visualized. The study underlines the importance of employing multimodal imaging to advance the knowledge about retinal cellular phenotypes in X-linked cone opsin mutations.

10. • Pedersen HR, Hagen LA, Landsend ECS, Gilson SJ, Utheim OA, Utheim TP, Neitz M, Baraas RC: **Color vision in aniridia**. *Invest Ophthalmol Vis Sci* 2018, **59**:2142-2152.

Pedersen *et al.* examined the association between color vision and retinal structure in persons with congenital aniridia revealing the tight link between central retinal development and visual function.

11. •• Pedersen HR, Neitz M, Gilson SJ, Landsend ECS, Utheim OA, Utheim TP, Baraas RC: **The cone photoreceptor mosaic in aniridia: within-family phenotype-genotype discordance**. *Ophthalmol Retina* 2019, **3**:523-534.

Pedersen *et al.* used multimodal AOSLO as well as OCT imaging to assess variability in cone numerosity and foveal hypoplasia structure in several members of the same family who had aniridia caused by deletion in the *PAX6*. The results showed considerable variability in cone density within each grade of foveal hypoplasia and underlines the importance of advancing knowledge about retinal cellular phenotype in aniridia.

12. Neitz J, Neitz M: **The genetics of normal and defective color vision**. *Vis Res* 2011, **51**:633-651.

13. Carroll J, Dubra A, Gardner JC, Mizrahi-Meissonnier L, Cooper RF, Dubis AM, Nordgren R, Genead M, Connor TB Jr, Stepien KE *et al.*: **The effect of cone opsin mutations on retinal structure and the integrity of the photoreceptor mosaic**. *Invest Ophthalmol Vis Sci* 2012, **53**:8006-8015.

14. Wagner-Schuman M, Neitz J, Rha J, Williams DR, Neitz M, Carroll J: **Color-deficient cone mosaics associated with xq28 opsin mutations: a stop codon versus gene deletions**. *Vis Res* 2010, **50**:2396-2402.

15. • Patterson EJ, Wilk M, Langlo CS, Kasilian M, Ring M, Hufnagel RB, Dubis AM, Tee JJ, Kalitzeos A, Gardner JC *et al.*: **Cone photoreceptor structure in patients with x-linked cone dysfunction and red-green color vision deficiency**. *Invest Ophthalmol Vis Sci* 2016, **57**:3853-3863.

Patterson *et al.* used multimodal AOSLO imaging to assess residual cone structure in participants with *OPN1LW* or *OPN1MW* opsin mutations associated with cone dysfunction and congenital red-green colour vision deficiencies to elucidate the genotype-phenotype link. The results revealed a direct link between cone mosaic disruption and *OPN1LW/MW* opsin mutations and underlines the importance of advancing knowledge about retinal cellular phenotype in cone dysfunctions.

16. Baraas RC, Hagen LA, Dees EW, Neitz M: **Substitution of isoleucine for threonine at position 190 of s-opsin causes s-cone-function abnormalities**. *Vis Res* 2012, **73**:1-9.

17. Dubis AM, Cooper RF, Aboshiha J, Langlo CS, Sundaram V, Liu B, Collison F, Fishman GA, Moore AT, Webster AR *et al.*: **Genotype-dependent variability in residual cone structure in achromatopsia: toward developing metrics for assessing cone health**. *Invest Ophthalmol Vis Sci* 2014, **55**:7303-7311.

18. Cideciyan AV, Hufnagel RB, Carroll J, Sumaroka A, Luo X, Schwartz SB, Dubra A, Land M, Michaelides M, Gardner JC *et al.*: **Human cone visual pigment deletions spare sufficient photoreceptors to warrant gene therapy**. *Hum Gene Ther* 2013, **24**:993-1006.

19. Langlo CS, Patterson EJ, Higgins BP, Summerfelt P, Razeen MM, Erker LR, Parker M, Collison FT, Fishman GA, Kay CN *et al.*: **Residual foveal cone structure in cngb3-associated achromatopsia**. *Invest Ophthalmol Vis Sci* 2016, **57**:3984-3995.

20. Lee DJ, Woertz EN, Visotcky A, Wilk MA, Heitkotter H, Linderman RE, Tarima S, Summers CG, Brooks BP, Brilliant MH *et al.*: **The henle fiber layer in albinism: comparison to normal and relationship to outer nuclear layer thickness and foveal cone density**. *Invest Ophthalmol Vis Sci* 2018, **59**:5336-5348.

21. Sweatt JD: **The epigenetic basis of individuality**. *Curr Opin Behav Sci* 2019, **25**:51-56.

22. Sabesan R, Hofer H, Roorda A: **Characterizing the human cone photoreceptor mosaic via dynamic photopigment densitometry**. *PLoS One* 2015, **10**:e0144891.

23. Dees EW, Dubra A, Baraas RC: **Variability in parafoveal cone mosaic in normal trichromatic individuals**. *Biomed Opt Express* 2011, **2**:1351-1358.

24. Dees EW, Gilson SJ, Neitz M, Baraas RC: **The influence of l-opsin gene polymorphisms and neural ageing on spatio-chromatic contrast sensitivity in 20-71 year olds**. *Vis Res* 2015, **116**:13-24.