Cone opsins, colour blindness and cone dystrophy: Genotype-phenotype correlations

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X-linked cone photoreceptor disorders caused by mutations in the *OPN1LW* (L) and *OPN1MW* (M) cone opsin genes on chromosome Xq28 include a range of conditions from mild stable red-green colour vision deficiencies to severe cone dystrophies causing progressive loss of vision and blindness. Advances in molecular genotyping and functional analyses of causative variants, combined with deep retinal phenotyping, are unravelling genetic mechanisms underlying the variability of cone opsin disorders.

S Afr Med J 2016;106(6 Suppl 1):S75-S78. DOI:10.7196/SAMJ.2016.v106i6.11001

My interest in inherited eye disease was initiated at the genetic outreach clinics conducted by the University of Cape Town (UCT) Department of Human Genetics at schools for visually and hearing-impaired children in the Western Cape. As a clinical registrar from 1992 to 1998, I accompanied Prof. Peter Beighton on numerous clinics, where I developed a fascination for understanding the genetic mechanisms underlying the sensory deficits affecting many of the children and their families. This led to a research initiative into the molecular genetics of profound childhood deafness in South Africa, which became the topic of my PhD thesis. Prof. Beighton is an inspirational teacher and one of the first clinical genetics lectures he gave to medical students at UCT began with a whirlwind tour of genetic conditions around the world. One of the conditions he described was the X-linked eye condition Bornholm eye disease; and another, a severe autosomal form of colour blindness affecting the Indonesian islanders of Pingelap, whose disorder, achromatopsia, was caused by an early settler who carried a rare recessive founder mutation. So it was, with a sense of déjà vu, that over 20 years later at the University College of London (UCL) Institute of Ophthalmology, UK, I started a research project to evaluate the role of the cone opsins in a panel of British male patients at Moorfields Eye Hospital, London, with a variety of retinal eye conditions ranging from Bornholm eye disease to X-linked incomplete achromatopsia (blue cone monochromacy) and X-linked cone dystrophy. I present a synopsis of X-linked cone opsin disorders and review recent work on their phenotypes and genotypes. It gives me great pleasure as a former PhD student of Prof. Beighton's to write this article in his honour.

Background

Mutations in the human long wavelength- and middle wavelength-sensitive cone opsin genes cause several X-linked cone vision defects including red-green colour blindness (MIM 303800, MIM 303900), X-linked cone dysfunction (MIM 300843), blue cone monochromacy (BCM; MIM 303700) and X-linked cone dystrophy (COD5; MIM 303700).[1-5] These allelic X-linked cone opsin disorders display wide inter- and intrafamilial variability. However, in the majority the visual defect is non-progressive. A few, conversely, show signs of deterioration with increasing visual impairment, macular atrophy, retinal pigmentation and electroretinographic (ERG) dysfunction of S cones and rods.[4-9] In these it appears that the presence of dysfunctional opsin results in degeneration of the photoreceptor. Recent studies have shown that cone opsin disorders, with specific visual and retinal phenotypes, are caused by several different genetic mechanisms.[10-12] As a result, genotype-phenotype correlations are beginning to emerge that influence predictions of the course and severity of disease.

X-linked cone photoreceptor disorders

The photoreceptor layer of the retina is composed of a mosaic of L (red), M (green) and S (blue) cones, interspersed by rods. Cones are most abundant in the central retina and are responsible for colour vision, daylight vision and high visual acuity, while rods are responsible for peripheral and low-light vision. Normal human trichromatic colour vision requires three functioning classes of cone, each characterised by a constituent photopigment with a specific range of light sensitivity. Photopigments are comprised of an opsin protein linked to a light-sensitive chromophore and the spectral sensitivity of the pigment is determined by the amino acid sequence of the opsin.[1] Stimulation of the photopigment generates an electrical signal that is processed with signals from all three cone types before the combination is interpreted as trichromatic colour perception in humans. Loss of one cone type results in the dichromatic colour vision disorders protanopia (loss of functional L cones) and deuteranopia (loss of functional M cones).

Both the L and M cone opsin genes (*OPN1LW* and *OPN1MW*) are located in a head-to-tail arrangement on chromosome Xq28, and loss of both L and M cone types causes X-linked BCM, alternatively known as incomplete achromatopsia.[1,4] Affected individuals have severe loss of colour vision and visual acuity from birth, frequently with photophobia and nystagmus. In young subjects, BCM can appear similar to X-linked cone dystrophy (XLCD), an allelic disorder that shares many clinical features with BCM, but which has a later onset of symptoms and a progressive course.

Bornholm eye disease (BED; MIM 300843) is an X-linked cone dysfunction disorder characterised by myopia, astigmatism, impaired visual acuity, optic nerve hypoplasia, abnormal retinal pigmentation and reduced cone ERG responses.[13] Originally described in a large kindred from the island of Bornholm in Denmark with deuteranopia, and later in several families with protanopia, this type of cone dysfunction is associated with loss of function mutations in the cone opsin array.[2,3,14]

XLCD is a heterogeneous condition characterised by loss of colour vision, impaired visual acuity, macular atrophy and ERG

evidence of severe disturbance of L and/or M cone function, often with additional S cone and occasional rod involvement. XLCD is most often associated with mutations of the retinitis pigmentosa GTPase regulator gene (*RPGR*) on chromosome Xp11.4. However, several large XLCD kindreds were found to be negative for mutations in *RPGR*. Linkage analysis and subsequent candidate gene screening in one of these families revealed a novel missense mutation in a highly conserved residue (*c.529T>C*; *p.Trp 177Arg*) in exon 3 of both L and M opsin genes. Interestingly, the variant was found within a region of identical sequence in the two genes, indicating that it had been transferred from the M gene to the L gene by means of a partial gene conversion, a process whereby sequence is transferred from one gene to another without any change occurring in the donor sequence.[5]

Molecular genetics of the cone opsin gene array

OPN1LW and *OPN1MW* are duplicated genes that arose from a single opsin primate ancestor. The genes retain 98% sequence similarity and their close proximity and nose-to-tail genetic arrangement results in frequent meiotic mis-pairing and non-homologous recombination, and less commonly, in gene conversion events. This genetic predisposition to structural rearrangement has led to the introduction and maintenance of a great variety of color vision phenotypes, both normal and deficient, in modern-day humans.

The L and M opsin genes in the ancestral opsin array lie adjacent to one another with a single L gene lying upstream of one or more M genes (Fig. 1). The number of genes in the arrays of modern humans varies considerably, however, only the first two genes are expressed in the retina. A mature photoreceptor expresses only one opsin, and the gene that is selectively expressed determines the L or M destiny of the photoreceptor. The selection process is driven by an upstream regulatory sequence or locus control region (LCR) that interacts with specific promoters of the first or second gene in the array.[1]

The L and M opsin genes have six exons. Exons 1 and 6 are identical between L and M genes with all sequence variation occurring in exons 2 - 5. In exon 5, there are seven amino acids that vary and these help to define the opsin, because two residues (at positions p.277 and p.285) are responsible for most of the spectral difference between L and M pigments. In exons 2, 3 and 4 there are eleven sites at which the amino acids can vary as a result of recombination between the ancestral L and M genes. Four of these are dimorphisms (at positions p.116, p.180, p.230 and p.233) that affect the absorption spectra of the opsin, while the remainder are individually believed to be functionally and visually neutral.

Genetic mechanisms of X-linked opsin disorders

Stable red-green colour vision deficiency with normal visual acuity is common, affecting ~8% of males.[15] Often this occurs as a consequence of a complete or partial deletion of either the L or M gene, leaving only a single functional gene to be expressed in all cones that, in a normal individual, would have been destined to be L or M; hence the difficulty in differentiating colour in the red/green spectrum. In phenotypes where a colour vision disorder is associated with loss of visual acuity, a number of causative mutations have been identified and classified according to their mechanism of action.

The first mechanism, a common cause of BCM, involves large-scale deletions of the LCR, which result in a complete absence of expression of both L and M cone opsin.[4]

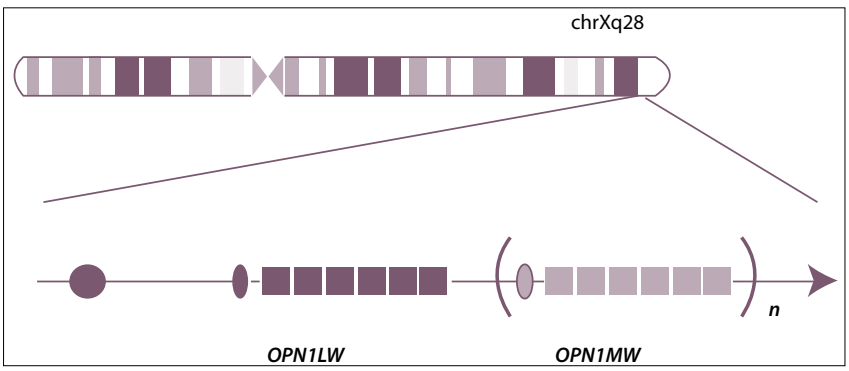

*Fig. 1. Schematic of the wild type L (*OPN1LW*) and M (*OPN1MW*) opsin gene array on chromosome Xq28. The L and M genes are arranged in tandem with the L gene in a 5' position to one or more (n=1 - 6) M genes. The genes have 6 exons (boxes). Interaction of the LCR (black circle) with the promoter (oval) determines gene expression.*

A second mechanism involves inactivation of one or more genes in the array by a missense mutation causing an amino acid change, or a partial deletion. Opsins with missense mutations are expressed, but some, including the common pathogenic variant *c.607T>C p.Cys203Arg*, are mis-folded and become mis-localised within the cell, resulting in an inability to bind the chromophore. The *p.Cys203Arg* mutation disrupts a critical di-sulphide bond that causes the protein to mis-fold, forming a non-functional opsin.[16] Similarly, the missense mutation *p.Trp177Arg* that causes XLCD, also mis-folds and is retained in the endoplasmic reticulum.[5] Toxic aggregation of the mis-folded mutant protein may be the cause of photoreceptor cell death and a degenerative phenotype.

Other mutant cone opsins could also have the potential to mis-fold. In a molecular study of BCM, a family was reported in which the affected males had a single hybrid gene with a deletion of exon 2. The deletion was not predicted to cause a frame shift in the protein and it would therefore be unlikely to undergo nonsense mediated decay (NMD). Instead, misfolding and toxic aggregation of the mutant protein could explain the progressive phenotype in this family.[9]

A third mechanism that has recently been recognised as a significant cause of X-linked opsin disorders involves the unexpected deleterious effects of specific combinations in exon 3 of normal polymorphisms that arose by genetic recombination between the L and M opsin genes. One such combination of polymorphisms was first noted by Nathans as the only change in a family with BCM, but it was not initially thought to be significant.[4] This family had a set, or haplotype, of common DNA variations (Table 1) in exon 3 that would normally translate to a series of amino acids p.[153Leu; 171Ile; 174Ala; 178Val; 180Ala]. This haplotype is commonly referred to as LIAVA, a term comprising the first letters of each variant amino acid in the series. This same haplotype was subsequently associated with XLCD and BCM in several studies.[17-19] A second rare haplotype of nucleotide variants (Table 1) translating to p.[153Leu; 171Val; 174Ala; 178Val; 180Ala] or LVAVA, has also been associated with XL cone dysfunction,[20] BCM and with cone dystrophy.[10,12,14] However, we now know that it is not the amino acid changes that are causing disease but the combinations of nucleotides. The single nucleotide variants comprising the LIAVA and LVAVA haplotypes are common (MAF >0.05 among

Table 1. Exon 3 reference and IC haplotypes

		Exon 3 DNA haplotypes, coding nucleotide variant and corresponding amino acid positions							
Opsin/ Exon 3 haplotype	**Opsin function**	**c.453 p.151**	**c.457 p.153**	**c.465 p.155**	**c.511 p.171**	**c.513 p.171**	**c.521 p.174**	**c.532 p.178**	**c.538 p.180**
L opsin	Normal	g	c	g	g	g	c	a	t
LVAIS			L		V		A	I	S
M opsin	Normal	a	a	c	g	g	c	a	g
MVAIA			M		V		A	I	A
IC opsin	Affected	g	c	g	a	t	c	g	g
LIAVA			L		I		A	V	A
IC opsin	Affected	g	c	g	g	g	c	g	g
LVAVA			L		V		A	V	A
IC opsin	Affected	a	a	c/g	a	t	c	g	g
MIAVA			M		I		A	V	A

individuals with colour normal vision) and are therefore not thought to be individually deleterious. However, in combination, they are associated with a loss of opsin function. The mechanism of disease caused by these rare haplotypes was unknown until recently, when it was discovered that the nucleotide combination resulting in an LIAVA haplotype interferes with normal recognition by the splicing mechanism in opsin pre-mRNA of exon 3. This causes it to be skipped in the resulting transcript.[21] The pathogenicity of exon 3 haplotypes was further established in a detailed study of BCM and XLCD phenotypes and genotypes in a British cohort of patients.[12] In this study, 41% of the affected males were found to have an unusual exon 3 haplotype, indicating that as a group, these haplotypes are a relatively common cause of opsin disorders and more frequent than either missense mutations or LCR deletions. LIAVA and LVAVA haplotypes were both found in the cohort in addition to a novel haplotype, MIAVA. These rare combinations of exon 3 nucleotides (Table 1), associated with disease, are collectively referred to as interchange (IC) haplotypes. In a functional investigation of the three IC haplotypes using a splicing assay, all three combinations of nucleotide polymorphisms were shown to interfere with normal pre-mRNA splicing. There were differences in exon skipping between the three haplotypes, and in the relative amount of transcript produced, suggesting different haplotypes cause different splicing patterns. The aberrant transcripts are predicted to result in a codon frame-shift and generation of a premature stop codon in exon 4 that would result in a truncated polypeptide, if expressed.However, the aberrant transcripts are likely to be removed by NMD.

Genotype-phenotype correlations

Although there is considerable phenotypic variation among subjects, observations from several studies indicate that subjects with LCR deletions are more likely to have congenital or very early onset of poor vision, nystagmus and myopia, with normal fundi and little evidence of progression.[10,12] Subjects with *p.Cys203Arg* also display congenital loss of colour vision and most are stable, but some older individuals show evidence of macular changes. Subjects with *p.Trp177Arg* had a more gradual onset of visual disturbance but developed extensive macular dystrophy and cone photoreceptor loss. Individuals with IC haplotypes also had a later onset of visual disturbance but were generally more progressive, showing macular changes after 40 years of age and dysfunction of both cone and rod systems on ERG.

Retinal imaging using high-definition optical coherence tomography and adaptive optics scanning light ophthalmoscopy shows distinct differences in the effects of LCR, missense and IC haplotype mutations on the integrity of the retinal layers and the structure of the cone mosaic.[10,11] While all mutations resulted in reduced retinal thickness and disruption of the photoreceptor mosaic, there were distinct patterns in the distribution and extent of the disruption between genotypes. Those with predominantly stable phenotypes (LCR deletions and *p.Cys203Arg*) had largely preserved lamination of the retina with disruption confined to a focal section only of the inner segment (Ise) layer, which corresponded to the S cone free zone of the fovea. Structural changes were specific to the L and M cones only. In contrast, subjects with progressive phenotypes (including exon 2 deletion and IC haplotypes) had widespread retinal thinning and disruption of cone structure, particularly in the fovea, with loss of L, M and S cones. Interestingly, despite severe foveal disruption, the IC haplotypes had better cone structure in the parafovea than the missense mutants, indicating that at least some L and M cones remain viable in these conditions. Of the IC haplotypes, LIAVA appears to be the least disruptive, showing normal inner retinal thickness and no change in cone structure in one subject over a period of 8 years.

Discussion

Variation in the clinical manifestation of the cone opsin disorders is well recognised but only recently have advances in genetics and imaging started to reveal underlying mechanisms of disease and to enable genomic subclassification of X-linked opsin disorders. The IC haplotypes illustrate an unexpected genetic mechanism that appears to be a frequent cause of severe cone dysfunction in both monochromatic (BCM) and dichromatic (BED) colour vision disorders.[12,14] The effects of these haplotypes on splicing depends on the combination of variants, so some may be degenerative while others are not. LVAVA has a progressive visual phenotype that is reflected in additional damage to the S cones and rods neighbouring mutant L/M cones. In contrast, retinal imaging of LIAVA suggests it may not result in the progressive loss of L/M cones (or it occurs much more slowly).[10] The difference may be related to the variable splicing effects of the haplotypes; LIAVA produces only a single aberrant transcript that is likely to undergo NMD, causing a congenital loss of cone function. LVAVA and MIAVA result in the production of several transcripts, one of which is normal opsin.[12] This could explain the later onset of visual impairment in some haplotypes and also the presence of a small remaining population of parafoveal L and M cones on retinal imaging. Although the

precise cause of photoreceptor degeneration is not understood, the retinoid byproducts of the visual cycle are cytotoxic. It is possible that the cones expressing the IC mutants remain viable through young adulthood but cannot operate normally in the visual cycle. This could lead to accumulation of toxic retinoids, affecting not only the cones expressing the mutant opsin, but also parafoveal S cones and rods.

Description of mutation-specific retinal phenotypes is particularly pertinent now that advances in gene replacement therapy are beginning to make prospects of restoration of cone function a reality. The genotypes with the greatest degree of cone preservation are the best candidates for intervention. In IC haplotypes, macular degeneration would limit the therapeutic time frame at later stages of the disease. However, strategies may be developed to slow the degenerative effects of these mutations. It will be important to assess the progressive nature of the various IC mutations and others to better determine the therapeutic potential in these individuals.

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