Eye (2007) 21, 1344–1351

www.nature.com/eye

CAMBRIDGE OPHTHALMOLOGY SYMPOSIUM

# Molecular genetics of infantile-onset retinal dystrophies

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**Abstract**

**Over the last decade there have been major advances in our understanding of the molecular pathology of inherited retinal dystrophies. This paper reviews recent advances in the identification of genetic mutations underlying infantile-onset inherited retinal disorders and considers how this knowledge may lead to novel therapeutic approaches.**
*Eye* (2007) **21,** 1344–1351; doi:10.1038/sj.eye.6702843

*Keywords:* retina; inherited; paediatric; gene

## Introduction

The inherited retinal disorders are historically classified according to natural history (stationary or progressive), the mode of inheritance (autosomal dominant (AD), autosomal recessive (AR), X linked (XL), or mitochondrial), and principal site of retinal dysfunction (pigment epithelium, rod or cone photoreceptor, or inner retina). Such classification involves careful history and examination, and detailed psychophysical and electrophysiological assessment. Such a method of subdividing retinal disease is however unsatisfactory as it may not reflect the molecular pathology of the retinal dysfunction. Advances in molecular genetics of retinal disease have allowed a more precise classification based on the genetic mutations underlying the disorders.

Rapid advances have been made in the field of retinal molecular genetics since the discovery of causative genes began in 1989. The database at Retnet (http://www.retnet.org) lists over 30 identified genes of inherited retinal dysfunction not including the syndromic forms of retinal dystrophies. Some of these genes encode proteins with a well characterised function in the retina, for example phototransduction, but other genes encode either novel proteins or proteins that have hitherto not been suspected to play a role in retinal function. The discovery of these genes has highlighted the role of new biological pathways in retinal structure and function. The identification of the genes underlying inherited retinal disease is an important first step in understanding the mechanism of retinal dysfunction and in developing effective treatment. It also has a more immediate impact on clinical management in improving diagnosis and genetic counselling.

This review aims to discuss the various infantile-onset retinal dystrophies including achromatopsia, blue cone monochromatism (BCM), congenital stationary night blindness (CSNB), and Leber's congenital amaurosis (LCA).

## Achromatopsia

Achromatopsia is a genetically heterogeneous group of AR stationary retinal disorders in which there is an absence of functioning cones in the retina.[1] Affected individuals have reduced central vision, poor colour vision, photophobia, pendular nystagmus, and usually normal fundi. Achromatopsia may occur in complete (typical) and incomplete (atypical) forms.

Complete achromatopsia (rod monochromatism) has an incidence of approximately 1 in 30 000. The disorder is inherited as an AR trait. The usual presentation is with early infantile-onset of nystagmus, poor visual acuity, and sensitivity to light. Vision is improved in mesopic conditions. Pupil reactions are slow and may show paradoxical responses (pupillary dilation to bright light). A hypermetropic refractive error is common. The nystagmus often improves with age, as can the photophobia.[2] Fundus examination is generally normal; however, macular atrophy may occasionally be present. Adults with this disorder usually achieve a visual acuity

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Received: 12 March 2007
Accepted: 22 March 2007

of 6/60 to 6/36 and have no true colour vision, although affected individuals may be able to distinguish primary colours using brightness clues. Rod-specific ERGs are normal but there are no detectable cone-derived responses.[3] Psychophysical testing reveals that retinal function is determined by rod photoreceptors alone.

Incomplete achromatopsia is best used to describe individuals with AR disease where the phenotype is a variant of complete achromatopsia. Individuals with this variant have better acuity (6/24 to 6/60) and retain some residual colour vision.[4]

Three achromatopsia genes have been identified to date, *CNGA3*, *CNGB3*, and *GNAT2*. All encode components of the cone photo-transduction cascade. Mutations in all three genes have been reported in association with complete achromatopsia,[5–8] whereas only mutations in *CNGA3* have been identified in incomplete achromatopsia.[9]

*CNGA3* and *CNGB3*, encode the $\alpha$- and $\beta$-subunits of the cGMP-gated (CNG) cation channel in cone cells. The cGMP levels are high in cone photoreceptors in the dark, which enables binding to the $\alpha$- and $\beta$-subunits of CNG channels. This permits an open channel conformation, cation influx, and cone depolarisation. At photopic levels, activated photopigments initiate a cascade producing increased cGMP-phosphodiesterase activity. As a result, the level of cGMP is reduced in the photoreceptor, which leads to closure of CNG cation channels and cone hyperpolarisation. Mutations in *CNGA3* and *CNGB3* account for the majority of cases of achromatopsia[10–12] There are over 50 disease-causing mutations in *CNGA3* that have been identified in patients with achromatopsia[13,14] with the majority being missense sequence variants. The *CNGA3* gene is highly conserved in evolution and it appears that there is little tolerance for substitutions with respect to the function of the channel polypeptide. Four mutations (Arg227Cys, Arg283Trp, Arg436Trp, and Phe547Leu) account for approximately 40% of all mutant CNGA3 alleles.,[15] By comparison, approximately only 12 mutations have been identified in CNGB3,[16–18] with the majority being nonsense variants. The most frequent *CNGB3* mutation to date, a 1 base-pair frameshift deletion 1148delC (Thr383fs), accounts for 80% of CNGB3 mutant disease chromosomes.[19,20]

A third gene, *GNAT2*, which encodes the $\alpha$-subunit of cone transducin, has also been implicated in achromatopsia.[21,22] In cone cells, light-activated photopigment interacts with transducin, a three subunit guanine nucleotide binding protein, stimulating the exchange of bound GDP for GTP. The *GNAT2* mutations result in premature translation termination and in protein truncation. *GNAT2* mutations are thought to be responsible for less than 2% of patients affected with achromatopsia.

The three genes,*CNGA3*, *CNGB3*, and *GNAT2* together account for the majority of cases of achromatopsia, but it is likely that there are one or more genes that account for a minority of cases. Uniparental isodisomy of chromosome 14 has been reported in association with an achromatopsia like phenotype[23] suggesting that there may be an additional achromatopsia gene on chromosome 14. However, recently a homozygous mutation in *CNGB3* has been identified in the original patient with the chromosome 14 rearrangement indicating that it is unlikely that there is a further locus on chromosome 14.[24]

### Blue cone monochromatism

Blue cone (S-cone) monochromatism affects less than one in 100 000 individuals; it is characterised by absence of L and M cone function.[25] There is normal rod and short wavelength sensitive cone function . BCM presents in infancy with reduced visual acuity, pendular nystagmus, and photophobia.[26] The nystagmus often reduces with time. Affected individuals are usually myopic and best-corrected visual acuity is usually in the range of 6/24 to 6/60. Fundus examination is usually normal, but macular atrophy is seen in some older individuals. BCM may be distinguished from achromatopsia by the mode of inheritance, the presence of a myopic rather than hyperopic refractive error and by the results of detailed psychophysical and electrophysiological testing. The photopic ERG is profoundly reduced in both disorders, but the S cone ERG is normal in BCM.[27] Psychophysical testing in BCM shows evidence of normal S-cone function in comparison to rod monochromats where there is either completely absent cone function or in incomplete forms some residual L- or M-cone function. In clinical practice it is important to use colour vision tests that probe the tritan colour axis as well as the protan and deutan to distinguish RM and BCM; residual tritan discrimination suggests the latter diagnosis.[28,29]

The normal human visual system compares the rate of quantum catches in three classes of cones; the short (S) wavelength sensitive, middle (M) wavelength sensitive, and long (L) wavelength sensitive to light at 430, 535, and 565 nm respectively. The L (red) and M (green) pigment genes are located on the X chromosome and the S cone (blue) pigment is encoded by a gene located on chromosome 7.[30] The L and M opsin genes consist of a tandem array of two or more repeat units of 39 kb on chromosome Xq28 that are 98% identical at the DNA level[31,32] Mutations in the L and M gene array underlie the molecular pathology of BCM.[33] Such mutations are of two main types. In the first group, the locus control

region (LCR) (which is common for both normal L and M pigment gene arrays), upstream of the L pigment gene is deleted. The deletion abolishes transcription of all genes in the pigment gene array and inactivates L and M cones.[34] In the second group of mutations, the LCR is preserved, but changes within the L and M pigment gene array lead to loss of functional pigment production. The commonest genotype is a single inactivated L/M hybrid gene. The first step in this second mechanism is unequal crossing over reducing the number of genes in the array to one, followed in the second step by a mutation that inactivates the remaining gene. A thymine to cystosine transition at nucleotide 648, resulting in a cysteine to arginine substitution at codon 203 (Cys203Arg), is the most frequent inactivating mutation.[35] This change disrupts the folding of cone opsin molecules via an absent disulphide bond between two extracellular opsin loops.[36] A third molecular genetic mechanism has been described in a single family of BCM where exon 4 of an isolated red pigment gene has been deleted.[37] Approximately 40% of blue cone monochromat genotypes are due to a one-step mutational pathway that leads to deletion of the LCR, with the remaining cases comprising a heterogeneous group of multistep pathways.[38–43] A minority of subjects are not found to have disease-causing changes to the opsin array, which raises the possibility that there may be a further genetic mechanism causing this disorder.

### Congenital stationary night blindness

CSNB is characterised by variable non-progressive visual loss, night blindness, and usually normal fundi, although some patients have pale or tilted optic discs. Inheritance may be AD, AR or XL with XL inheritance being most common. In XL and AR patients, the clinical presentation is usually in infancy with nystagmus, moderate to high myopia, strabismus, reduced central vision, and occasionally paradoxical pupil responses.[44] In contrast AD disease usually presents with nyctalopia but normal visual acuity; this subtype will not be considered further here.[45]

XL CSNB is subdivided clinically into complete and incomplete forms. Both subdivisions demonstrate a negative ERG, with a selective reduction in the inner nuclear-derived b-wave, so that it is smaller than the a-wave. In complete CSNB, the rod-specific ERG is usually non-recordable.[46] Cone ERGs reveal subtle abnormalities consistent with ON bipolar pathway dysfunction. In incomplete CSNB, there is a detectable rod-specific ERG, and cone ERGs are more abnormal than in the complete form. This is due to the involvement of both ON and OFF bipolar pathways. XL and AR disease are very similar clinically and on ERG investigation.

Two genes, *CACNA1F* and *NYX*, have been implicated in XL CSNB. Incomplete CSNB is associated with mutations in *CACNA1F*. This gene encodes the retina-specific $\alpha_{1F}$-subunit of the voltage-gated L-type calcium channel; it is expressed in the outer and inner nuclear layer and the ganglion cell layer.[47,48] Most of the mutations reported are inactivating truncation sequence variants. The loss of functional channels impairs the calcium flow into photoreceptors, which is required to sustain neurotransmitter release from presynaptic terminals. The retina remains in a partially light-stimulated state owing to the inability to maintain transmembrane potentials across bipolar cells. Patients are therefore unable to respond to changes in light levels. Most XL CSNB is non-progressive, but in a Japanese family with a retinal disorder caused by a *CACNA1F* mutation, affected individuals developed progressive loss of visual function and eventually an unrecordable ERG.[49]

Mutations in *NYX* are associated with complete CSNB. The NYX gene encodes nyctalopin, a proteoglycan with leucine-rich repeats, which are thought to be essential for protein interactions.[50] Nyctalopin is additionally thought to be involved in the development and structure of the ON pathway.

One form of AR CSNB is associated with mutations in *GRM6*; this gene encodes the glutamate receptor mGluR6.[51] Rod and cone receptors mediate synaptic transmission to ON bipolar cell dendrites via this receptor. More recently, AR CSNB has been associated with homozygous or compound heterozygous mutations in *CABP4*, a member of the calcium binding protein (CABP) family.[52] CABP4 is located in synaptic terminals and directly associated with the C-terminal domain of the calcium channel Cav1.4. The $Ca^{2+}$ influx through Cav1.4 triggers the continuous release of glutamate from the photoreceptor synapse in the dark.[53]

### Leber's congenital amaurosis

LCA, first described by Theodor Leber in 1869,[54] is a severe, generalised retinal dystrophy that presents at birth or soon after with nystagmus and severe visual impairment. The disorder accounts for 3–5% of childhood blindness in the developed world[55] and has an incidence of 2–3 per 100 000 live births.[56] It is associated with non-recordable or substantially abnormal rod and cone ERG.[57,58] The pupils react sluggishly to light and, although the fundus appearance is often normal, abnormal retinal changes including peripheral white dots at the RPE level, macular atrophy, retinal pigmentation, and vascular attenuation may be seen. Other findings include the oculodigital sign, microphthalmos, enophthalmos, strabismus,

keratoconus,[59] high refractive error,[60] cataract, and optic disc swelling.

LCA is usually inherited as an AR disorder, and to date nine genes (*GUCY2D*,[61] *AIPL1*,[62] *RPE65*[63,64] *RPGRIP1*[65,66] *CRX*[67,68] *TULP1*[69–71] *CRB1*,[72] *RDH12*,[73,74] and *CEP290*[75]and a three further loci (LCA3,[72] LCA5,[76] and LCA9[77]) have been reported to cause LCA. Mutations in the same genes are also responsible for early childhood-onset severe rod–cone dystrophies, and the term early-onset severe retinal dystrophy (EOSRD) may be a better term to describe a group of disorders including LCA that present in infancy and early childhood. In addition, mutations in two other genes (*LRAT*[78] and *MERKT*[79]) are associated with a similar EOSRD. These genes account for 20–50% of LCA cases,[80] and the true extent of genetic heterogeneity in this condition remains an area of active research. The relationship between genotype and phenotype has been the subject of several recent reviews and therefore will not be considered further here.[80,81]

### Management of infantile-onset retinal dystrophies: current treatment and future therapies

There is currently no specific treatment for any of these rare infantile-onset retinal dystrophies. However, it is important that the correct diagnosis is made in order to provide accurate prognostic information, and to offer informed genetic counselling, and educational and occupational advice. It is also important to maximise the use of residual vision by the provision of appropriate spectacle correction and low vision aids. Photophobia is often a problematic symptom in cone dysfunction syndromes, and red tinted lenses have been suggested to improve comfort and vision.[82] Patients may report some improvement with magenta lenses in BCM, which prevent rod desaturation.[83] In complete achromatopsia, red lenses allow low luminous efficiency wavelengths to be transmitted to the rod photoreceptors.[84] However, red brown lenses are more effective in incomplete achromatopsia, which have a wider spectral transmission thereby preserving residual colour discrimination.[85]

Given the evidence from some animal models that exposure to light can accelerate photoreceptor cell loss,[86] it is prudent to avoid exposure to bright lights in children with progressive retinal dystrophy.[87] A number of possible therapeutic approaches have been suggested for LCA/EOSRD including gene therapy, stem cell treatment, retinal transplantation, and pharmacological approaches such as the use of growth factors or synthetic retinoids.[88–93] Gene therapy currently appears to be the most promising approach, and the first clinical trials are likely to take place in patients with EOSRD associated with *RPE65* mutations. Gene therapy has proved effective in both the LCA Briard dog,[91] which has a naturally occurring 4 base-pair deletion in the *RPE65* gene, and the RPE65$^{-/-}$ mouse.[88] In the dog model, eyes treated with subretinal injections of adeno-associated virus containing cDNA of canine RPE65 showed significant signs of improvement after treatment in all electrophysiological parameters, pupillometry, and behavioural testing.[91] Uveitic responses to the novel protein developed in 75% of trans-gene-treated eyes, but only one eye (8%) was refractory to treatment.[92] These results are grounds for optimism that gene therapy may be equally effective in children with retinal disease due to mutation in *RPE65.*

Retinal cell transplantation stem cell therapy and the use of neuroprotective agents are other potential therapies. They have the advantage that they are not mutation specific and, in the case of stem cell therapy, could be performed later in the disease process. Approaches to treatment of retinal dystrophies in animal models have included cell transplantation including ARPE19 cells,[94] Schwann cells,[95] brain-derived stem cells,[96] marrow-derived neural stem cells,[97] and retinal progenitor cells.[98] However, preventing tissue rejection and enabling host retinal integration remains a major challenge. Much basic scientific clinical research needs to be carried out before clinical trials of stem cells or transplantation can get underway.

Various neuroprotective substances, including neurotrophic factors, growth factors, cytokines, or combinations of these, have prevented or delayed photoreceptor cell loss in animal models.[99–104] The most promising of these is CTNF, which has been shown to slowdown photoreceptor degeneration in a large animal model.[105] CTNF may however induce unwanted changes in retinal structure and function leading to reduced ERG responses[106] This may limit the therapeutic effect of this approach in the longer term. Phase one clinical trials of CTNF in advanced retinitis pigmentosa in man have been completed.[107] In this study, human ciliary neurotrophic factor (CNTF) was delivered into the vitreous by cells transfected with the human CNTF gene. The cells were encapsulated in a semipermeable membrane and surgically implanted in the vitreous. There were no serious complications after 6 months of treatment. Phase 2 trials are underway and the results are eagerly awaited.

Much progress has been made in characterising the various forms of infantile-onset retinal dystrophies, and many of the causative genes have been identified; it is likely that the remaining genes will be identified over the next few years. There is increasing optimism that effective therapies will be developed for at least some of these severe disorders.

**Acknowledgements**

This work was funded by the European Union (FP6), EVI-Genoret, Foundation Fighting Blindness USA, and Special Trustees of Moorfields Eye Hospital. This paper is based on a presentation at the Cambridge Ophthalmological Symposium, September 2006.

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