ABSTRACT
Achromatopsia is a complex inherited retinal disease that affects the cone cell function. It is usually an autosomal-recessive disease and is characterized by pendular nystagmus, poor visual acuity, lack of color vision, and marked photophobia. CNGA3, CNGB3, GNAT2, PDE6C, PDE6H, and ATF6 gene mutations have been identified as associated with this disease. New diagnostic and therapeutic tools are being studied. Optical coherence tomography and fundus autofluorescence are important imaging techniques that provide significant information about the progression of the disease. The genetic approach for these patients is a current important issue and gene therapy is an ongoing therapeutic option already being studied in clinical trials. The purpose of this review was to survey the current knowledge on diagnosis and treatment options in achromatopsia. [J Pediatr Ophthalmol Strabismus. 2018;55(2):85-92.]

INTRODUCTION
Achromatopsia is an inherited retinal disease that affects the cone cell function. It is a genetic dysfunction of all types of cones1 and is usually an autosomal-recessive disease that affects 1:30,000 to 1:50,000 births. It is characterized by presentation at birth or early infancy with pendular nystagmus, poor visual acuity, lack of color vision, and marked photophobia.2,3

CLINICAL CHARACTERISTICS
The clinical signs and symptoms of achromatopsia vary. Typical clinical presentation includes photophobia, pendular nystagmus, poor visual acuity, and color vision deficiency, among others. These symptoms usually start at birth or early infancy and their severity varies depending on genetic and phenotypic variability. Other symptoms such as central scotomas or eccentric fixation can also be features of achromatopsia,2 which can be clinically classified as complete or incomplete depending on the intensity and presence of the different clinical symptoms.

Complete achromatopsia, also known as typical achromatopsia or rod monochromatism, presents during early infancy with photophobia, pendular nystagmus, and poor visual acuity (usually worse than 20/200). The nystagmus and photophobia may become less noticeable over time, but visual acuity remains stable.2 In incomplete achromatopsia, also known as atypical achromatopsia, this triad of symptoms may be less intense; in particular, visual acuity can reach up to 20/80.2 In these cases, one or more cone types may be partially functioning along with the rods.4

Fundus examination is usually unremarkable. Evidence of the disease is uncommon and may appear as the narrowing of blood vessels, retinal pigment epithelium disturbances (separated into three categories: presence of retinal pigment epithelium disturbance [50%], no retinal pigment epithelium

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disturbance [27.5%], and atrophy [22.5%]), or alteration of the foveal reflex\(^2\) (Figure 1).

**GENETICS**

Six genes have been associated with achromatopsia, five of which are related to components of the cone-specific phototransduction cascade and one of which is related to endoplasmic reticulum function.

The most commonly affected genes are \(CNGA3\)\(^6,7\) and \(CNGB3\)\(^8\), which encode for the \(\alpha\) and \(\beta\) subunits of the cGMP-gated cation channel, respectively. Mutations in these two genes set up to 80% of all patients with complete achromatopsia.\(^5\)

Mutations in the \(GNAT2\), \(PDE6C\), and \(PDE6H\) genes are less frequent, each accounting for less than 2% of achromatopsia cases. Recently, a novel achromatopsia disease gene (ATF6) was identified in patients with achromatopsia and normal cone phototransduction genes. The ATF6 gene is related to the endoplasmic reticulum function,\(^4,9,10\) which is related to the unfolded protein response.\(^11-13\)

Regarding the type of achromatopsia and genetic findings, Thiadens et al.\(^14\) found no relationship between clinical subtypes and the affected gene. Conversely, Aboshiha et al.\(^3\) found only the \(CNGA3\), \(CNGB3\), and \(GNAT2\) genes to be related to incomplete or atypical achromatopsia. Additionally, Kohl et al.\(^4\) noted the genes associated with incomplete achromatopsia to be \(CNGA3\), \(GNAT2\), and \(PDE6H\). As studied by Rosenberg et al.\(^15\) and Vincent et al.\(^16\) certain pathogenic variants in the \(GNAT2\) and \(CNGA3\) genes are associated with a mild phenotype of incomplete achromatopsia. Pathogenic variants in the \(PDE6H\) gene also lead to the incomplete form of achromatopsia.\(^17\)

**DIAGNOSTIC OPTIONS**

**Clinical Evaluation**

Clinical evaluation includes all of the above mentioned clinical characteristics.

**Family History**

The family history is helpful to orientate the diagnosis by the inheritance mode. Family trees are useful in these cases.

**Electrophysiology Tests**

The most common electrophysiology tests used in the diagnosis of achromatopsia are electroretinogram and visual-evoked potentials.\(^18,19\)

A full-field electroretinogram evaluates the whole retina. This technique sometimes fails to show pathologic changes in cases of achromatopsia due to a limited number of photoreceptors within the fovea compared to the entire retina. Genead et al.\(^20\) found that 75% of patients with achromatopsia showed normal responses in this test. Additionally, deficits in rod and rod-mediated function occur in primary cone dysfunction syndromes.\(^21\)

Typical full-field electroretinogram in achromatopsia would show an absence of cone response because there is an absence of functional cones (Figure 2).

A multifocal electroretinogram simultaneously records electroretinogram signals from different retinal locations, enabling the detection of small zones of retinal dysfunction. It is useful in detecting localized abnormalities in the macular, paramacular, or mid-peripheral retina not obvious on examination. A multifocal electroretinogram allows more precise diagnostic information and eliminates the contribution of the extramacular retina. The diagnosis of achromatopsia is supported when there is an absent response from a 30- or 15-Hz stimulus in the cone-driven pathway of the multifocal electroretinogram. However, this tool cannot differentiate between complete and incomplete achromatopsia.\(^20\)

A visual-evoked potential is a recorded occipital wave that evaluates the integrity of the visual pathway, particularly the optic nerve function because its dysfunction causes prolongation of the P100 latency. In
achromatopsia cases, some degree of cone-mediated visual-evoked potential response may be present; this is due to a cortical magnification effect from some functional cones present in the macular region.\textsuperscript{18,19} Therefore, this is often normal and its clinical importance lies in excluding other pathologies.

**Optical Coherence Tomography**

Optical coherence tomography (OCT) is a great tool for the imaging and study of different structures in the eye. In particular, the retina has been extensively studied with this technique. Achromatopsia has also been studied with macular OCT. In 2014, Sundaram et al.\textsuperscript{5} studied the foveal structure in achromatopsia and described five categories: (1) continuous inner segment ellipsoid layer (appeared in 22.5% of the patients studied); (2) ellipsoid layer disruption (27.5%); (3) ellipsoid layer absence (20%); (4) presence of a hyporeflective zone (22.5%); and (5) outer retinal atrophy including retinal pigment epithelium loss (7.5%). No differences in the age, visual acuity, contrast sensitivity, or retinal sensitivity were found between patients in these five groups.\textsuperscript{5}

Sundaram et al.\textsuperscript{5} also evaluated the presence of foveal hypoplasia, which is defined by the presence of one or more inner retinal layers in the central fovea. In this study, 52.5% of the patients had foveal hypoplasia that could be observed in the macular OCT images. There was no significant difference in age, contrast sensitivity, retinal sensitivity, or fixation stability in patients with and without hypoplasia. Additionally, visual acuity and reading acuity were surprisingly better in patients with evidence of foveal hypoplasia compared to those without,\textsuperscript{5} which has also been observed in patients without achromatopsia.\textsuperscript{22}

Greenberg et al.\textsuperscript{23} also proposed a staging system based on OCT images to facilitate the classification of the disease into different phases of progression that may have therapeutic implications. This system included five stages: (1) the outer retinal structure is intact, but there is flattening and subtle discontinuity of the inner segment ellipsoid line; (2) the inner segment ellipsoid line is disrupted; (3) the classic optically empty space is observed with absent photoreceptors in the fovea, but the retinal pigment epithelium appears intact; (4) the optically empty space is disrupted and the retinal pigment epithelium is partially disrupted; and (5) the retinal pigment epithelium is completely disrupted and the outer nuclear layer is lost.\textsuperscript{23}
Mean foveal and outer nuclear layer thicknesses at the fovea of patients with achromatopsia have been shown to be significantly lower than in those without achromatopsia. Varsányi et al. also described this feature in patients with achromatopsia; total macular volume and central retinal thickness were significantly reduced in comparison to healthy controls.

It has also been noted that there is no association between genotype and phenotype regarding retinal structure and function.

In Figure 3, we can observe an example of macular OCT in achromatopsia. Following the description of Sundaram et al., ellipsoid layer disruption can be observed, with central foveal thickness measured to be 199 µm and the outer nuclear layer measured to be 59 µm, which is less than that of healthy individuals. Following the staging system of Greenberg et al., this patient would be at stage 2. Macular hypoplasia can also be observed because more than one retinal layer can be observed over the fovea (Figure 3). In early childhood, foveal alteration has been reported to be more mild than in older individuals with achromatopsia, which suggests the need for early therapeutic intervention. Neither age nor genotype alone predicts the degree of photoreceptor damage. Accordingly, in anticipation of future gene therapy trials in humans, Yang et al. proposed spectral-domain optical coherence tomography (SD-OCT) to be an important tool for the early assessment and stratification of macular architecture in young children with achromatopsia.

Thomas et al. observed progressive longitudinal changes in retinal morphology in achromatopsia on SD-OCT. These changes showed that achromatopsia may be a progressive disorder and the implementation of gene therapy during the early stages of the disease may provide the best prognosis.

Thiadens et al. reported that cone loss observed in SD-OCT occurred in 42% of affected individuals who were younger than 30 years and up to 95% in patients older than 30 years. They concluded that gene therapy should be applied in the first decade of life.

There is no clear association of the disruption of retinal structure and function in achromatopsia with age, suggesting that the window for intervention by gene therapy is wider than previously thought. Therefore, the potential benefit could be better predicted by specific measurements of the photoreceptor structure rather than simply by age.

Sundaram et al. also studied the retinal structure and concluded that candidates for gene therapy intervention should be considered individually, irrespective of their age. Foveal hypoplasia should not be an exclusion criterion. They also observed that assessing the degree of residual cone structure by measuring the ellipsoid line intensity ratio might be useful in determining the suitability of potential trial participants.

Fundus Autofluorescence

Fundus autofluorescence is a relatively new imaging technique. Lipofuscin is a product of phagocytized photoreceptor outer segments that accumulate in the retinal pigment epithelium. When exposed to short- to medium-wavelength visible light, lipofuscin will autofluoresce. Fundus autofluorescence imaging takes advantage of the autofluorescent properties of lipofuscin to document its accumulation.

Fahim et al. described fundus autofluorescence in patients with achromatopsia. They observed that the fundus autofluorescence images of younger patients revealed foveal hyperautofluorescence, whereas those of older patients revealed a punched-out foveal hypofluorescence with discrete borders and varying degrees of surrounding hyperautofluorescence at times. This was also observed by Kohl et al., who commented that these lesions correlated with the lesion area in the OCT.

Color Vision Tests

Patients with achromatopsia demonstrate abnormalities in all three axes of color vision. However, testing of color vision may be unreliable because patients may begin to discern colors based on differences in brightness or learn associated object–color relationships. Some of these tests include the Rayleigh anomaloscope (which is the most reliable test), Farnsworth Munsell 100-Hue, and Panel D-15 tests.
Visual Fields

Visual field examination usually demonstrates relative central scotoma, but this test can be difficult to perform due to a lack of steady fixation.\(^2\)

Genetic Testing

Molecular genetic testing approaches can include serial single-gene testing, use of a multi-gene panel, and more comprehensive genomic testing.\(^4\)

**Serial Single-Gene Testing.** Serial single-gene testing is a targeted analysis for the most common pathogenic variants. The order for serial single-gene testing is based on the frequency of pathogenic variants in each gene.\(^4\)

**Multi-Gene Panel.** A multi-gene panel includes all of the achromatopsia-related genes and other genes that may be considered of interest.\(^4\)

**More Comprehensive Genomic Testing.** More comprehensive genomic testing includes targeted exome,\(^29\) whole-exome, and whole-genome sequencing. This may be considered if other tests have not confirmed the diagnosis.\(^4\)

**Importance of Genetic Testing.** Genetic testing can be useful for securing the diagnosis, risk assessment in relatives, prenatal diagnosis, and prognosis, and identifying carriers and new affected genes.\(^4\) Genotyping is a crucial exercise because human gene-specific clinical trials to study photoreceptor rescue will depend on the particular diagnosis. Additionally, it also allows for a more precise prognosis of the possible future clinical evolution. Because treatments are gene specific and the “window of opportunity” is time sensitive, accurate, rapid, and cost-effective genetic testing will play an ever-increasing crucial role.\(^30\)

**TREATMENT OPTIONS**

There is currently no cure for achromatopsia. Similar to filtered glasses and contact lenses, color recognizing devices, and other technological aids, treatments are usually focused on improving quality of life.\(^4\)

Gene therapy is currently being studied. Animal studies using adeno-associated virus therapy have shown great advances. Cone-targeted gene therapy has shown success in animal studies,\(^31-34\) in which the therapy improved cone survival and recovered cone electroretinogram amplitudes to near normal levels.

Another treatment option that is being studied is the use of neuroprotective compounds such as the ciliary neurotrophic factor, which has been shown to inhibit progressive degeneration of rod and cone photoreceptors in some animal models and clinical trials. It also improved cone electroretinogram function and vision in dogs with achromatopsia. However, success with therapy based on either genes or neuroprotective compounds would require the cone photoreceptors to be present and viable within the macula. Although there are still many difficulties to solve, stem cell–based therapy is being studied as a potential treatment of retinal degenerative diseases in which patients have already lost their photoreceptors.

**Therapies**

**Treatment of Manifestations.** The current standard of care consists of managing symptoms, including dark or special filter glasses or red-tinted contact lenses to reduce photophobia, which may also improve visual acuity and be useful in avoiding light damage to the retina\(^36\); low vision aids such as high-powered magnifiers for reading; and social considerations such as preferential seating in the front of the class or social group promotion.

**Gene Therapy.** Several strategies are currently being studied to restore gene function in cone disorders. A specific vector is always necessary to deliver the target gene into the cell because DNA alone is not able to complete the task. Vector choice depends on the size of the cDNA of the type of targeted cells, the relevant gene, stability of expression, and immunogenicity.\(^37\)

Adeno-associated viruses have been successfully used as vectors for gene therapy.\(^37\) However, restrictions of this technology are its limited cargo size, only enabling the transport of genes up to a specific size, and putative immune responses to the viral capsid that may also limit recurrent treatments.\(^38\)

The ability of viruses to introduce genetic material into cells has been used to target genetically affected cells. Modified viruses have been developed for this purpose and are known as viral vectors.\(^39\)

To transduce specific cells, the viral vector has to be introduced close to the targeted cell surface. For example, if retinal pigment epithelium or photoreceptors are the target cells, then the vector usually needs to be administered in a subretinal injection. If the ganglion cells are to be targeted, the intravitreal approach may be a good option.\(^39,40\) Future developments may allow the use of the intravitreal route to target the outer retina, avoiding the more difficult and potentially damaging subretinal injection.\(^39\)
Rescue of cone function structure using adenovirus-associated viral vectors has been achieved in animal models. Recently, three clinical Phase I/II safety trials for gene replacement therapy using adenovirus-associated viral vectors for achromatopsia association with CNGA3 and CNGB3 have been approved and are recruiting patients (https://clinicaltrials.gov/ct2/results?cond=Achromatopsia; NCT02610582, NCT02599922, and NCT03001310).

**Stem Cell Therapy.** Cell replacement therapies using retinal progenitor cells derived from embryonic or induced pluripotent stem cells show great promise in treating early onset degenerative diseases such as cone disorders. In recent animal studies, it was observed that the transplanted cells that were differentiated into adult photoreceptor cells were positioned at the correct location and formed connections with bipolar cells, demonstrating the potential of this approach to rescue retinal function in future clinical trials.

**Pharmacological Approaches.** Gene replacement therapy is suitable for retinal disorders that result from mutations that cause a loss of function. However, we would need a different viral vector for each gene involved. For this reason, there is interest in developing other therapies. Treatments using neuroprotective agents, growth factors, and anti-apoptosis agents are being investigated. Such approaches would not restore function, but they would be useful when photoreceptors initially function and vision is lost as photoreceptor cells die.

The ciliary neurotrophic factor was recently shown to improve cone function in CNGB3 mutant achromatopic dogs and CNGB-/- mice. Unfortunately, in 2012, a Phase I/II clinical trial (NCT01648452) investigated the effects and safety of an intraocular implant releasing ciliary neurotrophic factor in five patients with achromatopsia and found no objectively measurable enhancement of cone function by assessments of visual acuity, mesopic increment sensitivity threshold, photopic electroretinogram, or color hue discrimination. Subjectively, some patients reported beneficial changes, including reduced light sensitivity and aversion to bright light, but slowed adaptation to darkness, consistent with ciliary neurotrophic factor action on rod photoreceptors.

In response to this publication, Liu and Varnum suggested that this lack of response to treatment might be due to few residual cones failing to produce a detectable change in function. They suggested using high-resolution quantitative retinal imaging techniques for the selection and evaluation of the results of the treatment.

Pharmacological approaches are particularly suited for disorders caused by a gain of CNG channel function, which can be due to an increase in cGMP affinity. One such mutation in CNGA3 (N471S) that was associated with complete achromatopsia was analyzed in Xenopus oocytes and it was demonstrated that cGMP affinity. This opens up the possibility for these types of products to be used as potential treatments for retinal diseases due to overactive CNG channels.

**When to Treat**

One of the challenges for gene therapy is the limited time period to achieve a successful treatment outcome. The progression rate of the different diseases may affect the window of opportunity for treatment. Therefore, treatment opportunities may be explained by determining the viability of cone cell bodies using SD-OCT and adaptive optics. However, in patients with early onset cone disorders, gene augmentation therapy should ideally start as early as possible.

Lee et al. suggested that achromatopsia is a continuously altering and progressive process in the developing retina. With human gene therapy trials imminent, their results suggest that therapy should be considered at early ages while the photoreceptors are still developing, thereby potentially facilitating normal retinal maturation.

Thomas et al. observed for the first time in SD-OCT that there were progressive longitudinal changes in retinal morphology in achromatopsia. These changes showed that this is a progressive disorder and implementation of gene therapy during the early stages of the disease may provide the best prognosis.

Dubis et al. studied criteria to assess residual photoreceptor integrity in achromatopsia. They presented cone reflectivity as a measure that can be used to characterize cone integrity in achromatopsia. Cone numerosity and/or density combined with cone reflectivity could be used to estimate the therapeutic potential. They suggested that these measurements could be a more immediate indicator of efficacy than behavioral measures, which may take longer to change.
The lack of a clear association with age suggests that the window of opportunity for intervention by gene therapy is wider in some individuals than previously thought. Potential benefits should be better predicted by specific measurements of the photoreceptor structure.\(^5\)

**FUTURE PERSPECTIVES**

**Identification of Genes Associated With Cone Disorders**

Whole-exome sequencing combined with homozygosity mapping or linkage analysis has proven to be successful in the discovery of novel retinal disease genes.\(^50\) In the coming years, whole-exome and whole-genome sequencing will facilitate the identification of novel gene defects.\(^26\)

Large patient cohorts should be studied through collaborative efforts. Additionally, the causality of novel variants can be tested through cellular and animal model studies.\(^37\)

**Transcriptomics**

The knowledge of naturally occurring splice events may aid the interpretation of RNA analysis of known retinal disease genes in patients. It has been observed that only approximately 35% of the genes involved in retinal dystrophies are expressed at high enough levels in lymphoblastoid cells to study the transcriptome from an individual’s blood sample.\(^37\)

Photoreceptor precursor cells can be differentiated from a patient’s keratinocytes through the generation of induced pluripotent stem cells.\(^37\) These can be generated from adult somatic cells and can subsequently be differentiated into retinal cells.\(^31\) RNA analysis of those cells may enable the detection of splice defects that might not be detectable in lymphoblastoid cells.\(^37\)

**Alternative Cone Rescue Strategies**

Cell replacement therapies using retinal progenitor cells derived from embryonic or induced pluripotent stem cells are being studied. These cells could be valuable in cell-based therapies.\(^42\) In a study by Gonzalez-Cordero et al.,\(^42\) the transplanted cells showed differentiation into adult photoreceptor cells, positioning at the correct location, and formation of connections with bipolar cells, demonstrating the potential of this approach to rescue retinal function in future clinical trials.

**CONCLUSIONS**

Achromatopsia is a complex inherited disease. New diagnostic and therapeutic tools are being studied. OCT and fundus autofluorescence are important imaging techniques that provide significant information about the progression of the disease. Gene therapy is an ongoing therapeutic option already being studied in clinical trials, making the genetic approach of these patients a current important issue.

**REFERENCES**


