

Gene Therapy for the Treatment of Achromatopsia: Recent Advances

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Abstract

Achromatopsia (ACHM) is a rare autosomal recessive group of cone dysfunction syndrome. The typical clinical presentation includes colour vision defect, early onset of visual impairment, pendular nystagmus and severe photophobia. There is currently no cure for ACHM, although novel interventions are under investigation including gene therapy. The aim of this article is to provide clinicians with an up-to-date comprehensive literature review, concerning the clinical considerations, genetic findings that are characteristic to ACHM and management strategies with particular interest for gene therapy. We describe gene therapy in animal models and clinical trials in humans.

Keywords: *Achromatopsia (ACHM); Gene Therapy*

Introduction

Complete achromatopsia (ACHM, other synonyms: rod monochromatism, achromatism, total color blindness, pingelapese blindness) is a rare retinal disorder from birth/early infancy, characterized by complete or severely reduced color blindness, photophobia, pendular nystagmus of variable degree (which can improve with age), and severely reduced visual acuity usually 20/200 - 20/400 due to dysfunction of all three types of cones (S, M, and L) with no response on photopic electroretinogram (ERG), while rod function is preserved although defects in rod photoreceptor function have also been reported [1-3]. Ocular fundus may also be normal, but atrophy of the retinal pigment epithelial cells may be present [1-3]. In ACHM has been demonstrated reorganization in the visual pathways with cone-dependent pathways monopolized by rods, which justifies a better and faster dark adaptation in patients with achromatopsia [4]. The prevalence of ACHM ranges from 1 in 30,000 to 1 in 50,000 live births around the world [1-3]. Family history can be useful as ACHM displays an autosomal recessive inheritance pattern, but often the affected individual is the only affected in a large family. Consanguinity increases the likelihood of an autosomal recessive disease (known as pseudo-dominance). In the past, ACHM has been considered a stationary disease but recent studies have shown structural progression in several patients with increased age [3,5]. In this update, we will review recent advances in clinical symptoms, genetics, and management of ACHM, especially focused on gene therapy.

Method of literature search

The literature was searched using PubMed references, Genetics Home Reference-NIH and ClinicalTrials.gov. We also searched the internet database for genetics of retinal diseases, the portal GeneReviews® and the genetic database Online Mendelian Inheritance in Man (OMIM-NCBI). Pertinent articles from the English and non-English literature were selected. In addition, relevant references contained in those articles were included in this review.

Genetic and phenotypic heterogeneities

There are six genes associated with ACHM (Table 1) [3,6,7]. CNGA3 (*ACHM2*, OMIM # 216900) (2q11.2) and CNGB3 (*ACHM3*, OMIM # 262300) (8q21.3) account for around 75%-80% of all ACHM cases (50% caused by mutations in the CNGB3 gene, the most prevalent in Northern European patients, and a 25% by mutations in the CNGA3 gene, more prevalent in the Middle East and China) [8,9]. CNG (cyclic nucleotide-gated) channels are in all cone photoreceptors outer segment and play a key role in phototransduction cascade controlling the membrane potential of photoreceptors [6,7]. CNG forms a heterotetrameric complex composed in a stoichiometric ratio of 3:1 subunits of CNGA3 and CNGB3 respectively [10,11]. CNGB3 gene codes for the beta-subunit of cone cyclic-GMP gated cation channels. It was originally identified in the Pingelapese people of the Eastern Caroline Islands [12]. A missense mutation (serine x phenylalanine, S435F) results in a defective β subunit [12]. The most prevalent mutation (T383fsX) is a null mutation that results in a non-functional truncated protein that does not transit properly to the cell membrane [13,14]. The majority of mutations in the CNGB3 gene result of nonsense mutations [9]. CNGA3 gene codes for the alpha-subunit of cone cyclic-GMP gated cation channels [10]. More than 50 mutations have been described in CNGA3, the majority being missense mutations [15,16]. GNAT2 (*ACHM4*, OMIM # 613856) (1p13.1) is associated with complete achromatopsia (< 2%) and codes for the alpha-subunit of cone transducin [17]. Transducin is involved in the signal transduction cascade [17]. More than 10 GNAT2 mutations have been identified [18]. Achromats with GNAT2 mutations have better preserved cones compared with CNGB3 or CNGA3 achromats [19]. PDE6C (*ACHM5*, OMIM # 610024) (10q23.33) is identified as a cause of achromatopsia (< 2%) and codes for the cone cGMP-specific 3',5'-cyclic phosphodiesterase subunit α gene [20]. PDE6H (*ACHM6*, OMIM # 610024) (12p12.3) encode the inhibitory γ -subunits of the cone-specific cGMP phosphodiesterase gene. It is responsible in less than 1% of cases and the three different cones are not affected to the same extent with S cone function more preserved [21]. Variants in ATF6 (activating transcription factor 6A) (*ACHM7*, OMIM # 605537) (1q23.3) was recently identified as a novel genetic cause of ACHM [22]. ATF6 is a key regulator of the unfolded protein response and cellular endoplasmic reticulum homeostasis localized throughout the retina particularly in the inner retinal layers. It is responsible in 1%-2% of cases [22]. Complete-ACHM, also known as rod monochromatism, is generally considered to lack cones and have vision worse than 20/200. These patients can not perceive any color vision.³ Complete achromat may show the Flynn phenomenon (constriction of the pupil in the dark). This phenomenon is not unique to ACHM [23]. Incomplete-ACHM, also known as atypical-ACHM or incomplete rod monochromatism, may have slightly better visual acuity in the range of 20/80 - 20/200. Incomplete achromats may have some residual color vision when the light is just right because a small residual cone functioning is present [3,24]. In these patients adaptive optics showed that cone inner segments was retained, but outer segments were disrupted [5]. The use of intravitreal ciliary neurotrophic factor (CNTF) injections may have a role in these cases [25]. Achromats usually have high hyperopic refractive errors although hyperopia and myopia were equally frequent in CNGA3 achromats, while myopia was more frequent than hyperopia among CNGB3 achromats [24,26]. ATF6 gene has a key role in human foveal development [22]. Spectral-domain optical coherence tomography scans showed foveal hypoplasia and variable disruption of the ellipsoid zone at the fovea could be a mark of ATF6-related phenotype [3,22]. About half of CNGB3 ACHM have some degree of foveal hypoplasia (Figure 1) [25]. The association between these genes mutations, photoreceptor integrity and ACHM phenotype are not appear to directly correlate [27] but recently a study established the first evidence of gene mutation and residual cone integrity in ACHM [19].

Gene	OMIM #	Locus Site	Inheritance pattern	Code
CNGA3	OMIM # 216900	8q11.2	ar	Alpha-subunit of cone cyclic-GMP gated
CNGB3	OMIM # 262300	2q21.3	ar	Beta-subunit of cone cyclic-GMP gated
GNAT3	OMIM # 613856	1p13.1	ar	Alpha-subunit of cone transducin
PDE6C	OMIM # 610024	10q23.33	ar	cGMP-specific 3',5'-cyclic phosphodiesterase subunit α
PDE6H	OMIM # 610024	12p12.13	ar	Inhibitory γ -subunits of the cone-specific cGMP phosphodiesterase
ATF6	OMIM # 605537	1q23.3	ar	Activating transcription factor 6A

Table 1: Genes in achromatopsia.

ar: Autosomal Recessive.

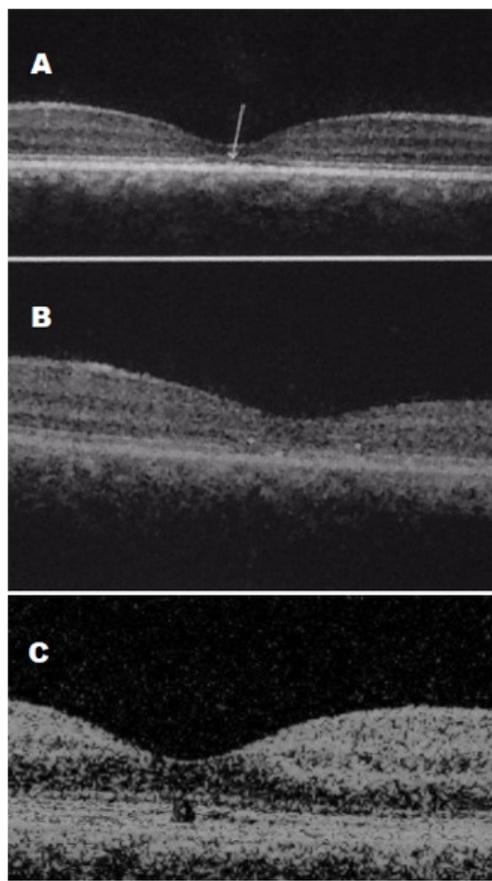


Figure 1: SD-OCT, morphological alterations of outer retinal layers at the fovea: variable degrees of disruption of inner segment ellipsoid (A-arrow- B-stars), an optically empty cavity or hyporeflective zone at the base of the fovea (C).

Therapeutic options

At this moment, no treatments have been approved for ACHM, only symptomatic therapy as deep red tinted glasses or contact lenses can reduce photophobia and low vision aids to improve visual acuity [28]. Selective filters may enhance the achromat's ability [28]. A number of novel interventions are under investigation including gene therapy [3,29]. Several approaches to gene therapy, including replacement gene therapy, optogenetics, addition of a growth factor, suppression gene therapy, and gene editing (engineered or bacterial nucleases), have been considered to treat people with vision-threatening inherited ophthalmological diseases [3,29]. The eye is an ideal area for gene therapy because it is a small organ needing minimal amount of vectors, relatively easy to see inside, and it is one of a few sites with immune privilege provided by the blood-ocular barrier [3,30]. This means less likely of inflammatory response than in other parts of the body [3,30]. How does gene therapy work? Gene therapy is the treatment of a disease by replacing a missing or defective gene responsible for the disease [29]. The most common type of gene therapy is replacement of a non-functional copy of a gene introducing a normal copy but is only possible when the genetic defect is identified (CNGB3, CNGA3) and when a gene delivery system is not a limiting

factor [29]. This type of therapy works best in autosomal recessive diseases and when viruses (carriers or vectors) can be injected directly into the target site or cells (cones) [31]. A gene carried into the eye via an injection into the vitreous (similar to an intravitreal injection) or a more invasive subretinal injection in the operating room. Subretinal delivery of the vector is the method of choice, targeted most efficiently with less viral doses and limited inflammation [31] but with possible surgical complications such as thinning of the central retina, macular hole and retinal detachment [31] (Figure 2). Originally planned as a treatment of a variety of inherited disorders, gene therapy is now applied to non-inherited diseases like tumors or much more common diseases like macular degeneration [3,31]. The transfected gene is expressed, resulting in functional protein causing the cell to return to a normal state and restored cone photoreceptor function. Cone cortical activity has been shown after gene therapy in a canine model of ACHM [32].



Figure 2: Picture showing the technique of subretinal delivery of viral vector..

Vectors systems, a limiting factor?

The goal of gene therapy is to introduce new genetic material into target cells without toxicity to non-target tissues [33]. Vector is a vehicle that is used to deliver a healthy gene to compensate for or correct abnormal gene. Vector systems can be divided into viral vectors (engineered virus) (VV) and non-viral vectors (N-VV) [34-36]. Gene transfer with VV is called transduction while transfer via N-VV is called transfection [33]. VV has advantages over N-VV methods: transfer of the genetic material to the cells is more efficient and specific and even a single dose is sufficient [36]. VV can be divided into two types: integrating (retrovirus, lentivirus, and adeno-associated virus) and non-integrating into the human genome (adenovirus), so the new copy can be lost during cell division and the expression is transient (Table 2) [33].

VV	Adenovirus	AAV	Retrovirus	Lentivirus
Genome	DNA	DNA	RNA	RNA
Genome size	39 kb	5 kb	3-9 kb	3-9 kb
Tropism/Infection	Dividing/Non-dividing cells	Dividing/Non-dividing cells	Dividing cells	Dividing/ Non-dividing cells
Host Interaction	Non-integrating	Non-integrating	Integrating	Integrating
Transgene expression	Transient	Transient or stable	Long lasting	Long lasting
Packaging capacity	7.5Kb	4.5Kb	8Kb	8kb
Family	Adenoviridae	Parvoviridae	Retroviridae	Retroviridae

Table 2: A comparison of different VV in use for gene therapy in ACHM. VV: Viral Vector; AAV: Adeno-Associated Virus.

Viral vectors

Retro-virus

Retroviruses (Retroviridae family) are a class of RNA viruses that possess an RNA of about 10 Kb and the enzyme reverse transcriptase [37]. The genetic material of the virus is incorporated and has become part of the genetic material of the host cell; if this host cell divides later, its descendants will all contain the new gene [37]. The most common retrovirus used is derived from the murine leukemia virus. Retroviruses have been used for *ex vivo* gene therapy as they are unable to efficiently infect non-dividing cells [37].

Lenti-virus

Lentivirus (lenti- Latin for “slow” a genus from the Retroviridae family) has the ability to infect non-dividing cells, as such retinal neurons and pigment epithelium cells [38]. Lentiviruses deliver a significant amount of genetic information into the DNA of the host cell, so they are one of the most efficient methods of a gene delivery vector. Lentiviral transfer systems provide long-term expression and efficient transfer gene without inflammatory responses [33,36].

Adeno-associated virus

Adeno-associated (AAV) virus is a class of DNA viruses currently used in the majority of gene therapies [37]. AAV (parvovirus family) genome is small, only allowing about 4.8 kb of added DNA. AAV vectors lack all viral genes, including those that are responsible for integration into host chromosomes, further minimizing activation of immunity. To date, AAV has been shown to be safe and effective in preclinical and clinical settings. AAV vectors maintain persistent transgene expression over many years in postmitotic cells following a one-time administration of the vector [37]. Currently, recombinant AAV (rAAV) novel AAV-capsid serotypes (rAAV2/2[*MAX*]), AAV2 (Y444F), AAV2 (Y730F), AAV8 (Y733F) and AAV9 (Y446F) are powerful tools for transgene expression in the neurosensory retina demonstrating efficacy at lower doses and safety using vitreous (rAAV2/2[*MAX*]) [38] or subretinal [AAV2 (Y444F), AAV2 (Y730F), AAV2/8 (Y733F), AAV2/5 (Y719F) and AAV9 (Y446F)] delivery. All known ACHM genes have small sequences (< 2,600 bp), so AAV can be used as gene therapy vectors [39,40].

Adeno-virus

The genetic material of the adenoviruses is not incorporated into the human genetic material. As a result, this treatment will require re-administration in a target cell. Adenoviral vectors offer significant advantages: easy of purification and cell infection of various cell types, dividing or non-dividing [41]. The limitations: the short time of expression (range: 7 - 42 days) and viral genes are also transduced and expressed with an immune response [41]. Pre-existing antibodies can limit the effectiveness of this strategy, particularly upon a second exposure to the vector [41].

Risks factors associated with viruses mediated gene delivery must be considered: viruses can infect more than one type cell which triggered a massive immune response that could lead to organ failure. More recently designed vectors deliver specific genes to specific cells. The new gene could insert in a wrong location in the DNA, causing mutations or even cancer, especially retroviruses. Inserted genes could be over expressed, producing so much of the missing protein [42].

Attractive target: ACHM (primary cone channelopathy)

ACHM has been considered an ideal disease for gene therapy based on several key points:

1. ACHM has a relatively simple etiology arising from a single mutant protein [5].
2. Although of early onset, ACHM is considered a relatively stable disease with slow progressive non-functional cone loss and a wider window for gene therapy [3,22,28].

3. Animal models (small -mouse- and large -dog, monkey and sheep-) exist for the most prevalent genetic defects (CNGA3, CNGB3 and GNAT2) of the disease [3,43].
4. Rapid restoration of retinal function after treatment [3,43].
5. Recent success in retinal gene therapy in humans affected with Leber's congenital amaurosis indicate that it may be possible to restore cone function [43,44].

Animal models of ACHM

In monogenic diseases as ACHM, animal models are critical to develop therapies, especially gene replacement. Untreated animals with ACHM have the same signs of abnormal visual function as humans [45-50]. In animal models of ACHM genetic treatment has shown positive results to restore some cone function [45-50]. Treatment of animals (ACHM) with an AAV vector expressing the CNGB3 or CNGA3 protein resulted in long-term improvement in visual function, measured by ERG amplitudes and by observation of the ability to function in bright light before and after treatment [43,45-50]. Despite these positive results, use of different promoter and animal models providing improvements ranges from 10% to 90% [43]. In the human retina, the promoter is of great importance in gene therapy for ACHM. In animal models the recuperation of cone function is dependent of the age and promoter (PR2.1-promoter rescues cone function stable and remains permanent) [43,45-47].

Gene therapy for CNGB3 ACHM

Animal models

Small animal disease models

Murine model

A mouse model of CNGB3 achromatopsia, introducing a null mutation in the CNGB3 gene (*Cngb3*^{-/-}), manifests the same abnormal visual function like in human achromats patients, which is most dramatically manifested by daytime blindness especially under bright light conditions [45-47]. An AAV vector expressing a human CNGB3 gene rescued cone function (ERG amplitudes of up to 90% of normal) and improved visual behaviour in 6 months old *Cngb3*^{-/-} model of achromatopsia with a single subretinal injection [46-48].

Large animal disease models

Canine model

An inherited form of ACHM occurs among dogs, similar to humans with achromatopsia. The disease has been linked to a missense mutation in exon 6 (*CNGB3*^{m/m}) or a null mutation (*CNGB3*^{-/-}) [46-48]. Subretinal administration of AAV vectors encoding human CNGB3 gene led to long-term increased day vision and restored cone specific ERG amplitudes [46-48].

Primate model (*Cynomolgus macaques*)

A subretinal injection of an AAV vector expressing the human CNGB3 gene was safe and well tolerated.

Gene therapy clinical trials

1. Applied Genetic Technologies Corporation (AGTC; Gainesville, FL) is currently conducting a non-randomized, open-label, Phase I/II study of the safety and efficacy of rAAV2tYF-PR1.7-hCNGB3 administered to one eye by subretinal injection in individuals with CNGB3 ACHM (ClinicalTrials.gov Identifier: NCT02599922). The primary study endpoint will be safety and the secondary study endpoint will be efficacy. Subjects will be enrolled sequentially in four groups. Subjects in Groups 1, 2 and 3 will be at least

18 years of age and will receive a lower, middle or higher dose of study agent. Subjects in Group 4 will be at least 6 years of age and will receive the maximum tolerated dose identified in Groups 1, 2 and 3. Safety will be monitored by evaluation of ocular and non ocular adverse events and hematology and clinical chemistry parameters. Efficacy parameters will include visual acuity (changes in best corrected visual acuity compared to pre-treatment), light discomfort testing (changes in light discomfort testing compared to pre-treatment), color vision (changes in color vision testing compared to pre-treatment), static visual field, ERG, adaptive optics retinal imaging and OCT. Estimated primary completion date: December 2018 and estimated study completion date: December 2022. (Safety and efficacy trial of AAV gene therapy in patients with CNGB3 achromatopsia. Available at: <https://clinicaltrials.gov>).

2. Long-term follow-up study of participants following an open label, multi-centre, phase i/ii dose escalation trial of a recombinant adeno-associated virus vector for gene therapy of adults and children. This study is a longer-term follow-up study to collect data on longer-term safety and efficacy (ClinicalTrials.gov Identifier: NCT03278873). This study is recruiting at the time of writing.
3. Gene therapy for achromatopsia CNGB3. an open label, multi-centre, phase i/ii dose escalation trial of a recombinant adeno-associated virus vector (AAV2/8-hCARp.hCNGB3) for gene therapy of adults and children with achromatopsia owing to defects in CNGB3 (ClinicalTrials.gov Identifier: NCT03001310). This study is underway at the time of writing. (Available at: <https://clinicaltrials.gov>).
4. CNTF Implants for CNGB3 Achromatopsia (CNTF-CNGB3-1).

In six patients with CNGB3 achromatopsia that received intraocular implants that released ciliary neurotrophic factor no improvement in cone function occurred. (ClinicalTrials.gov Identifier: NCT01648452).

Gene therapy for CNGA3 ACHM

Animal models

Small animal disease models

Murine model

There are two mouse models of CNGA3 ACHM [49]. One of mouse models is a wild CNGA3 mutation, named *cpf15* (cone photoreceptor function loss type 5). The other was generated by introducing a null mutation into the CNGA3, *Cnga3*^{-/-}. Both models mimic the clinic of human patients and a single subretinal injection of an AAV vector expressing the mouse CNGA3 gene led to and delayed cone degeneration and increased cone responses on ERG amplitudes [43].

Large animal disease models

Canine model

Two wild CNGA3 ACHM models have been characterized: p.R424W (identified in German shepherd) a missense mutation with complete loss of cone function, and p.V644del (identified in Labrador retriever), a deletion mutation with failure of normal CNGA3 subunit assembly.

Sheep model

Awassi sheep model has been linked to a mutation in the CNGA3 gene. In these sheep (*CNGA3*^{-/-}) a single subretinal injection of an AAV vector expressing either a mouse or human CNGA3 gene led to increased cone responses on ERG amplitudes and increased day vision

maintained for up to 3 years post-injection.

Gene therapy clinical trials

1. A Multiple-Site, Phase I/II, Safety and Efficacy Trial of AGTC 402, a recombinant adeno-associated virus vector expressing CNGA3, in patients with congenital achromatopsia caused by mutations in the CNGA3 gene. (ClinicalTrials.gov Identifier: NCT02935517) This will be a non-randomized, open-label, Phase 1/2 study of the safety and efficacy of AGTC-402 administered to one eye by subretinal injection in individuals with achromatopsia caused by mutations in the CNGA3 gene. The primary study endpoint will be safety and the secondary study endpoint will be efficacy. Estimated study completion date June 2023. (Available at: <https://clinicaltrials.gov>).
2. University Hospital Tubingen and Ludwig-Maximilian University of Munich are also sponsoring Safety and Efficacy of a Single Subretinal Injection of rAAV.hCNGA3 in patients with CNGA3-linked achromatopsia investigated in an exploratory, dose-escalation trial. (ClinicalTrials.gov Identifier: NCT02610582). Efficacy data (improvement in visual function) and patient reported outcomes will be investigated exploratively as well as retinal imaging. They are all secondary endpoints in this trial. Estimated study completion date October 2021. (Available at: <https://clinicaltrials.gov>).

Gene therapy for GNAT2 ACHM

Small animal disease models

Murine model

Two wild GNAT2 ACHM models have been described. The *Gnat2* (*c.518A>G*) mouse with a missense mutation in exon 5 of transducin gene that results in complete loss of cone function [50] and ALS/LtJ strain with *cpfl3* mutation that leads to cone dysfunction and the progressive loss of cone alpha-transducin [51].

Gene therapy for PDE6C ACHM

Small animal disease models

Murine model

Mouse *cpfl1* Mutant (cone photoreceptor function loss 1) was discovered in the recombinant inbred strain CXB-1. Mouse mutant shows absence and rapid degeneration of cone function as early as 3 weeks of age [20].

Gene therapy for PDE6H ACHM

Pde6h *-/-* mouse model, in contrast to human patients, shows preserved visual functions [52].

Gene therapy for ATF6 ACHM

Atf6 *-/-* mice have normal retinal morphology and function at a young age but develop rod and cone dysfunction with increasing age [43].

Conclusion

Because the incidence of achromatopsia is rare compared to other inherited retinal diseases, the amount of funding are limited but the ability of gene therapies to provide durable benefits justifies and increasing efforts toward making gene therapy part of our routine treatment. AAV- gene therapy is in clinical development for patients with achromatopsia caused by mutations in the CNGB3 or CNGA3 genes. Next, harmless virus will be used to deliver the healthy genes to the patient's cells with less systemic reactions. Gene editing will

play an increasing role. Other alternatives remove the use of viruses in eye gene therapy: delivered genes shell in fat molecules that can carry the therapeutic gene into the cell by being admitted through the cell membrane and electroporates the gen directly applying an electrical charge to the cell less than two minutes creating pores on the cellular membrane. The remarkable advances in genetics have opened an exciting world to ophthalmologists to offer people new treatments for vision-threatening retinal diseases. In the next decade, gene therapy will be a routine treatment offered to patients.

Disclosure

The author reports no conflicts of interest in this work.

Ethics Approval and Informed Consent

This review was based on previous studies and does not contain any study with animals or humans by the author. Therefore, informed consent was not necessary.

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