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REVIEW ARTICLE

Achromatopsia: Genetics and Gene Therapy

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Abstract
Achromatopsia (ACHM), also known as rod monochromatism or total color blindness, is an autosomal recessively inherited retinal disorder that affects the cones of the retina, the type of photoreceptors responsible for high-acuity daylight vision. ACHM is caused by pathogenic variants in one of six cone photoreceptor-expressed genes. These mutations result in a functional loss and a slow progressive degeneration of cone photoreceptors. The loss of cone photoreceptor function manifests at birth or early in childhood and results in decreased visual acuity, lack of color discrimination, abnormal intolerance to light (photophobia), and rapid involuntary eye movement (nystagmus). Up to 90% of patients with ACHM carry mutations in *CNGA3* or *CNGB3*, which are the genes encoding the alpha and beta subunits of the cone cyclic nucleotide-gated (CNG) channel, respectively. No authorized therapy for ACHM exists, but research activities have intensified over the past decade and have led to several preclinical gene therapy studies that have shown functional and morphological improvements in animal models of ACHM. These encouraging preclinical data helped advance multiple gene therapy programs for *CNGA3*- and *CNGB3*-linked ACHM into the clinical phase. Here, we provide an overview of the genetic and molecular basis of ACHM, summarize the gene therapy-related research activities, and provide an outlook for their clinical application.

Key Points

Achromatopsia (ACHM) is caused by mutations in one of six autosomal recessive genes and affects all aspects of daylight vision.

No therapy for ACHM has yet been approved, but several preclinical studies provided proof of concept for adeno-associated virus gene therapy.

Five clinical gene therapy trials are currently underway for *CNGA3*- and *CNGB3*-related ACHM.

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1 Introduction

1.1 Clinical Manifestation, Etiology, and Genetics of Achromatopsia

Achromatopsia (ACHM) is a rare genetic eye disease that is inherited in an autosomal recessive manner and affects approximately one in 30,000 people [1]. Unlike color blindness, in which mutations and rearrangements in the genes encoding the various cone photopigments affect only spectral sensitivity but not the main photoreceptor function [2], ACHM has grave consequences for all aspects of daylight vision mediated by the cone photoreceptors. Patients with ACHM have poor visual acuity, photophobia, and nystagmus and are not able to distinguish colors [3]. Nystagmus is often present at birth or manifests in early infancy. These symptoms are due to a primary functional defect of the cone photoreceptors that manifests in early infancy that is also reflected in a severely reduced or absent light-adapted electroretinogram (ERG) but a largely preserved scotopic ERG signal [4, 5]. In addition to this functional defect, many patients show varying degrees of morphologic changes in the cone-rich central (foveo-macular) part of the retina, ranging from loss of the outer segments of the cones to profound atrophy of the outer retina, including loss of

the retinal pigment epithelium [3]. Currently, six genes are linked to ACHM (Table 1). Up to 90% of ACHM cases are due to mutations in *CNGA3* (OMIM #216900) and *CNGB3* (OMIM #262300) [6–8], which encode the alpha and beta subunits of the heterotetrameric cyclic nucleotide-gated (CNG) channel [9]. ACHM is genetically well-characterized, and there is a high percentage of solved cases [10]. *ATF6* (OMIM #616517), *GNAT2* (OMIM #613856), *PDE6C* (OMIM #613093), and *PDE6H* (OMIM #610024) are the other known ACHM genes [11]. With the exception of *ATF6* [12], all other ACHM genes encode essential components of the signal transduction cascade known as phototransduction (or visual transduction) (Fig. 1), which is responsible for converting the light signal into voltage and calcium signals [13]. The absence or dysfunction of any of these phototransduction genes results in functional impairment of the phototransduction cascade. *ATF6*, on the other hand, encodes a transmembrane transcription factor that is localized in the endoplasmic reticulum (ER), can activate the unfolded protein response, and plays a role in ER homeostasis [14, 15]. Most cases are associated with mutations in the two cone CNG channel genes, and mutations in the other known ACHM genes are much rarer and together account for less than 6–8% of cases [6, 11]. ACHM genes that have not yet been identified or unidentified mutations may be responsible for the remaining 5% of unexplained cases [10].

1.2 The Cone CNG Channel and its Role in Phototransduction

The cone CNG channel is made up of three CNGA3 and one CNGB3 subunits [13, 16]. Each subunit comprises six transmembrane segments, a pore region, a C-linker region, and a cyclic nucleotide-binding domain. Within the retina, the cone CNG channel is located exclusively in the plasma membrane of the outer segment of cone photoreceptors [13]. Instead of *CNGA3* and *CNGB3,* rod photoreceptors express the homologous genes *CNGA1* and *CNGB1*, which encode the corresponding alpha and beta subunits of the rod CNG channel. In both types of photoreceptors, the CNG channel is an integral part of the phototransduction cascade (Fig. 1). While the phototransduction cascades of cones and rods are similar, they share only a few common proteins, and most key proteins are encoded by distinct homologous genes, which co-evolved during evolution [17].

In both cases, the activity of the CNG channel is controlled by the levels of cyclic guanosine monophosphate (cGMP), which is the central second messenger of the phototransduction cascade. cGMP is produced by the receptor guanylyl cyclase (retGC), and its levels are balanced by the guanylyl cyclase-activating protein (GCAP) and phosphodiesterase (PDE) (Fig. 1). In the dark, high levels of cGMP keep CNG channels open, which conduct a mixed Na^+/Ca^{2+} current that maintains the cone cell in a depolarized state. This depolarization leads to continuous activation of synaptic voltage-gated calcium channels and release of glutamate from the cone synaptic terminal, transmitting the light signal to second-order neurons (bipolar and horizontal cells). The GCAP binds Ca^{2+} entering the outer segment through the CNG channel and inhibits retGC activity in a negative feedback mechanism, preventing excessive production of cGMP. Upon light exposure, photons hit the chromophores bound to the opsins (rhodopsin in rods and cone opsins in cones), triggering a conformational change and the release of the G-protein transducin, which activates the PDE to mediate hydrolysis of the second messenger cGMP [18]. The resulting drop in cGMP levels leads to closure of the CNG channel and hyperpolarization of the photoreceptor cell. This reduces the release of the neurotransmitter at the synaptic terminal, which is sensed by downstream glutamate receptors and triggers the transmission of the reverse signal to the brain.

Many inherited retinal disorders are caused by mutations in genes that encode proteins involved in the phototransduction cascades of rods and/or cones [19]. Although the phototransduction cascades of cones and rods are very similar in function and share some common proteins, many key proteins, although performing a similar function in the respective signaling cascade, are encoded by different genes and

Table 1 Overview of achromatopsia genes, animal models and preclinical studies

Gene	Chromosomal location	Phenotype/OMIM	Animal models	POC studies
ATF6	1q23.3	ACHM7/605537	KO mouse [12]	–
CNGA3	2q11.2	ACHM2/600053	KO mouse [66], cpfl5 mouse [91], ovine model [80]	[67, 80, 81, 83, 84, 96]
CNGB3	8q21.3	ACHM3/605080	KO mouse, mutant mouse [21], canine model [97], NHP model [51]	[75, 76, 89]
GNAT2	1p13.3	ACHM4/139340	Cpfl3 mouse [71]	[78]
PDE6C	10q23.33	Cone dystrophy 4/600827	Cpfl1 mouse [72]	–
PDE6H	12p12.3	ACHM6/601190	KO mouse [30]	–

KO knockout, *NHP* non-human primate, *OMIM* Online Mendelian Inheritance in Man, *POC* proof of concept

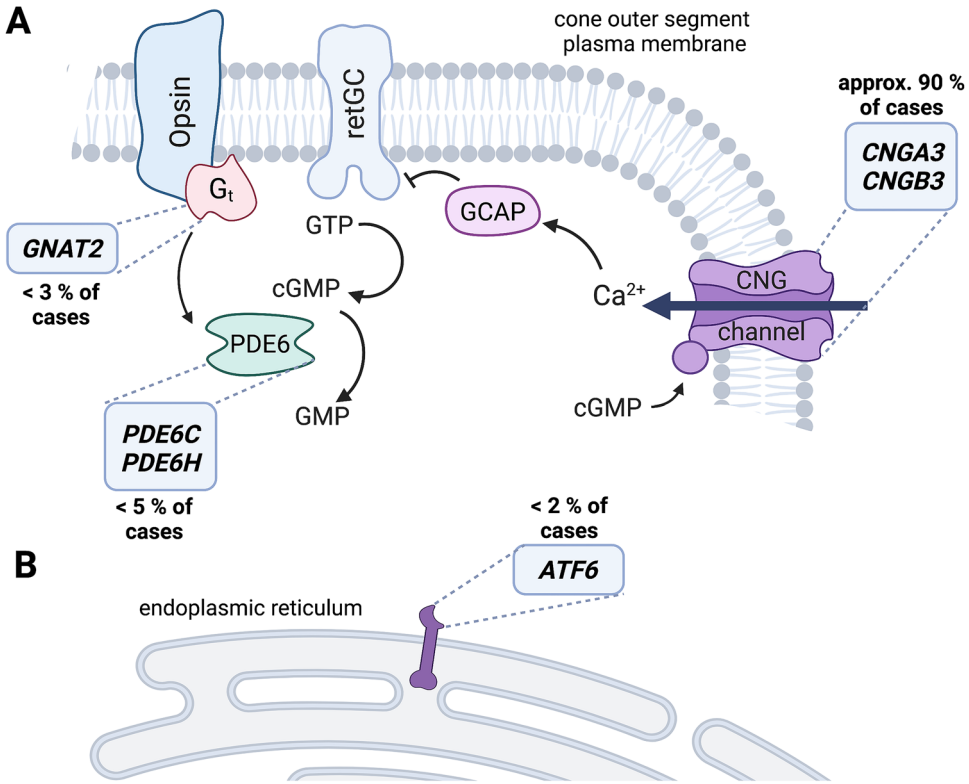

Fig. 1 Known achromatopsia (ACHM) genes and their functions. **A** Most genes associated with ACHM encode proteins involved in phototransduction. The most common ACHM genes are *CNGA3* and *CNGB3*, which encode the two subunits of the cyclic nucleotide-gated (CNG) channel, which is located in the plasma membrane of the outer segment. A less common disease gene is *GNAT2*, which encodes the cone transducin (G_t) that activates the phosphodiesterase (PDE6), which mediates the hydrolysis of the second messenger cyclic guanosine monophosphate (cGMP). The two genes encoding the cone PDE6 (*PDE6C* and *PDE6H*) have also been linked to ACHM. **B** The only known ACHM gene that does not encode a phototransduction cascade protein is *ATF6*. *ATF6* is localized in the endoplasmic reticulum and is involved in endoplasmic reticulum stress and the unfolded protein response. The relative frequencies of ACHM mutations in Europe and Northern America are given next to the boxes with the gene names. Created with BioRender.com. *GTP* guanosine-triphosphate; *retGC* receptor guanylyl cyclase

are expressed exclusively in the respective cell type (i.e., in only rods or only cones). Therefore, many inherited retinal diseases manifest in a primary functional defect of either the cone or the rod photoreceptors.

2 The Mutation Landscape in *CNGA3*- and *CNGB3*-Linked Achromatopsia

To date, more than 100 mutations in *CNGA3* [20] and nearly 100 mutations in *CNGB3* [6] have been found to cause inherited ACHM in humans. All known mutations are inherited in an autosomal recessive manner, and only homozygous or compound heterozygous patients show the typical symptoms of ACHM, whereas carriers have normal vision. Interestingly, a digenic and triallelic inheritance pattern with mutations in both genes has recently been identified in a subset of patients with ACHM [21]. The individual prevalence of *CNGA3* or *CNGB3* variants varies geographically. It is highest on Pingelap Atoll in Micronesia, where color-blind people make up almost 10% of the small island's indigenous population. This is because a typhoon in the eighteenth century decimated the atoll's population, leaving behind a small group of survivors who passed on a particular mutation in the *CNGB3* gene (p.S435F) [22] to their descendants [23]. Of the two genes, *CNGB3* is the more common ACHM gene in Europe and the USA (estimated prevalence of ≥50%) [7, 8], whereas *CNGA3* is most common in the Middle East and China. Among Israeli and Palestinian patients with ACHM, mutations in *CNGB3* account for only 8% of ACHM cases, whereas mutations in *CNGA3* account for more than 80% [19, 24]. A prevalence of approximately 80% for *CNGA3*-linked ACHM has also been reported for Chinese cohorts [25, 26]. Another difference between the two genes concerns the pattern of mutations. The majority of *CNGB3* mutations are nonsense, frameshift, or splicing mutations that result in truncated or severely impaired channel proteins [27, 28]. In contrast, most *CNGA3* mutations are missense mutations

that affect only single amino acid residues of the protein [27, 28]. Folding, intracellular processing, and transport are thought to be impaired [29]. The effects of individual CNGA3 amino acid substitutions on CNG channel function have mainly been studied in vitro [21, 30–46]. Some insights have been gained, but the exact mechanisms linking CNGA3 amino acid substitutions to cone photoreceptor dysfunction and eventual degeneration are still not well understood.

Because cone photoreceptor function is strongly reduced or absent from early infancy in affected people, there is no true progression of clinical symptoms over time. However, animal studies and morphological data from patients with ACHM suggest progressive degeneration and loss of cones over time [47, 48]. It is believed that the principal development and morphology of cones in ACHM is initially similar to that of unaffected cones, and diseased cones begin to degenerate in young adulthood and are eventually lost through the induction of various cell death mechanisms [49–56]. Unfortunately, the disease mechanisms involved in cone degeneration are only partially understood, and there is a great need to better characterize the pathobiology in affected cone photoreceptors.

3 Animal Models of ACHM

There are several genetically modified and naturally occurring small and large animal models for ACHM [12, 21, 30, 51, 57–65] (Table 1). The first animal model developed was the *Cnga3* knockout (KO) mouse, which helped to establish the genetic basis of *CNGA3*-linked ACHM [66]. Genetic inactivation of *Cnga3* in mice results in nonfunctional cone CNG channels because CNGB3 cannot form functional CNG channels in the absence of CNGA3 [48, 66]. As a result, *Cnga3* KO mice show selective loss of cone-mediated light responses [66], accompanied by progressive degeneration and cell death of cones [48]. An early hallmark of cone degeneration is the strong accumulation of the second messenger cGMP, suggesting its involvement in the degeneration process [50, 67, 68] (Fig. 1). Loss of cone photoreceptors in the *Cnga3* KO retina progresses much more rapidly in the ventral and nasal (S cone-rich) portions than in the dorsal and temporal (M cone-rich) portions [48], suggesting that cone degeneration affects the M- and S-cones in different ways. This is in line with observations in other mouse models of inherited retinal disorders [69]. Several naturally occurring mouse, dog, and sheep models with mutations or deletions in the *Cnga3* gene have since been identified that exhibit the typical features of ACHM and reduced cone function [58, 59, 62, 63].

There are also small and large animal models of *CNGB3*-associated ACHM [21, 57, 60, 61, 64, 65, 70]. Deletion of *Cngb3* in mice or dogs results in severely reduced, but not absent, cone function and progressive degeneration of cone photoreceptors [60, 64]. The remaining cone function in *Cngb3*-deficient animal models can be attributed to residual irregular homomeric CNGA3 channels that are transported to the outer segment in the absence of CNGB3 protein. Animal models also exist for the other less frequent ACHM genes [12, 30, 57, 71, 72]. All these animal models are very valuable for studies on the pathobiology of ACHM and for testing the efficacy of potential (gene) therapies. This is especially true for large animal models that better mimic the morphological features of the human eye. An example of this is the observation of macular morphological changes in the recently discovered non-human primate model of *PDE6C*-linked ACHM, which also exhibits features observed in patients with ACHM, such as foveal thinning and subtle bull's eye maculopathy [57].

4 Development of Gene Therapy for ACHM

Clinical management of ACHM currently includes only specialized genetic counseling, vision aids, and tinted contact lenses or glasses to alleviate the symptoms of photophobia [73]. However, there is no curative treatment for ACHM. The availability of suitable animal models and the advent of recombinant adeno-associated virus (rAAV) vectors as efficient and safe retinal gene transfer vectors [74] have facilitated the preclinical development of novel gene therapies for ACHM (Table 1). As described, ACHM is caused by mutations that are inherited in an autosomal recessive manner. Therefore, it is a very attractive candidate disease for so-called gene supplementation (or augmentation) approaches, which aim to transfer a healthy copy of the disease-causing gene into affected cells (in this case, cone photoreceptors). Several groups have developed gene supplementation therapies and tested them in relevant animal models of ACHM. Some of these approaches have already moved to the translational phase and are currently being tested in phase I/II clinical trials. The following sections summarize the key findings of the preclinical studies and provide an overview of the current status of the clinical trials.

5 Gene Supplementation Therapy: Preclinical Studies

Several preclinical studies have tested rAAV vectors with known tropism for retinal photoreceptors for gene supplementation in animal models of *CNGA3*-, *CNGB3*-, and *GNAT2*-linked ACHM [62, 75–78]. The first study to provide evidence for efficient gene supplementation to cone photoreceptors used the naturally occurring *Cpfl3* mouse model of *GNAT2*-linked ACHM [78]. These mice show

little or no cone-driven light responses and normal rod-mediated ERG responses. Subretinal injection of an rAAV vector expressing mouse *Gnat2* under the control of a human red opsin promoter resulted in increased light-adapted ERG responses and improved vision-guided behavior [78]. Despite the promising results, translation of this program is likely hampered by the low prevalence of *GNAT2*-linked ACHM, which accounts for less than 2% of ACHM [6].

More than a decade ago, rAAV-mediated *Cnga3* gene supplementation showed promising results in rescuing the ACHM phenotype in the well-characterized *Cnga3* KO mouse model, which lacks cone photoreceptor function from birth [77]. *Cnga3* KO mice treated after eye opening were able to generate cone-driven light responses despite being born without cone-mediated visual processing. Thus, this study provided the first evidence of sufficient plasticity of the visual system such that new functions transferred postnatally to previously nonfunctional cones could be properly processed in the mouse visual cortex to elicit biologically meaningful visual behavior. The treatment also had a positive effect on retinal morphology, normalized cGMP levels in cones, delayed cone cell death, and reduced the inflammatory response of Müller glial cells typical of retinal degeneration [48]. The therapeutic effect was also observed when treatment was initiated at a more advanced stage of the disease at 1 or 3 months of age and was stable for at least 12 months after treatment [79]. Similar therapeutic effects with subretinal *Cnga3* gene supplementation were reported from another study that used the naturally occurring *cpfl5* mouse model of *CNGA3*-ACHM [62, 80]. The studies described so far used subretinal delivery of rAAV vectors for *Cnga3* gene supplementation. Two other studies showed restoration of cone-mediated function in mouse models of *CNGA3*-ACHM after intravitreal delivery of engineered rAAV vectors that encoded *Cnga3* under control of a cone-specific promoter [81, 82]. If translatable, such novel rAAV vectors could be used in future studies to target a larger fraction of cone photoreceptors without the need to detach the central (foveomacular) part of the retina. Successful rAAV-based gene supplementation has also been described in the sheep model of *CNGA3*-ACHM [83]. Significant long-term improvement in cone function was demonstrated for at least 6 years after the one-time *CNGA3* gene supplementation treatment [84]. These promising preclinical studies led to the initiation of three independent CNGA3 gene therapy programs (Table 2). Safety studies in sheep and non-human primate models revealed some evidence of inflammation after subretinal *CNGA3* gene delivery but overall acceptable safety profiles for at least two different translatable *CNGA3* gene therapy products [85–88]. Safety data have not yet been published for the third *CNGA3* gene therapy product.

Restoration of cone-mediated vision by rAAV-based gene supplementation therapy has also been described in mouse and dog models of *CNGB3*-related ACHM [75, 76]. Initial studies used subretinal delivery of rAAV (AAV5 or AAV8) vectors expressing human *CNGB3* under control of cone-specific promoters and demonstrated long-term restoration of cone function and cone-mediated vision [75, 76]. Interestingly, the success rate of cone therapy in dogs was promoter and age dependent. Only the 2.1 kb human red opsin promoter and treatment at 28 weeks of age showed robust and sustained treatment effects [76]. Gene supplementation therapy also failed to restore normal visual acuity in older (6-month-old) *Cngb1*-deficient mice [75]. The exact reasons for the age dependence of treatment are not known but could be related to morphological changes observed in later stages of the disease. Interestingly, pretreatment with intravitreal ciliary neurotrophic factor (CNTF) allowed successful recovery of cone function and vision in 14- to 42-month-old *Cngb3*-mutant dogs [89]. The authors suggested that this was due to reversible CNTF-mediated shortening of outer segments and reduction of gene expression [89]. Based on these proof-of-concept studies, two independent translational programs for *CNGB3*-ACHM were initiated. Safety data obtained in mice, dogs, and cynomolgus monkeys were published from one of the programs [90–92]. The studies showed acceptable safety with vector- and dose-dependent inflammation and toxicity. Induction of

Table 2 List of currently ongoing or recently completed achromatopsia gene therapy clinical trials

Gene	Drug	Phase	Sponsor/company	NCT ID	Current status
CNGB3	AAV2tYF-PR1.7-hCNGB3	I/II	AGTC	02599922	Recruiting
CNGB3	AAV8-hCAR-hCNGB3 (Entacin-gene turiparvovec)	I/II LTFU	MeiraGTx/Janssen	03001310 03278873	Completed recruiting
CNGA3	AAV8-hCAR-hCNGA3	I/II IIb	RD-CURE	02610582	Completed recruiting
CNGA3	AAV8-hG1.7-hCNGA3	I/II	MeiraGTx/Janssen	03758404 03278873	Completed recruiting
CNGA3	AAV2tYF-PR1.7-hCNGA3	I/II	AGTC	02935517	Recruiting

LTFU long-term follow-up, *NTC ID* National Clinical Trial number of the ClinicalTrials.gov registry

neutralizing antibodies directed against the AAV capsid but not the *CNGB3* transgene was demonstrated in the sera of treated animals from all species tested [90–92].

6 Gene Supplementation Therapy: Clinical Studies

All of the aforementioned translational programs for *CNGA3*- and *CNGB3*-linked ACHM have already reached the clinical phase [9] (Table 1). The first clinical trial evaluated the effect of three different doses (1×10^{10} to 1×10^{11} total vector genomes per eye) of AAV8.CNGA3 in nine patients with *CNGA3*-linked ACHM and has reported 1-year [93] and 3-year data [94]. Treatment involved a single subretinal injection into one eye, with the subretinal bleb covering the foveo-macular region. Despite this highly invasive delivery procedure, the treatment was well-tolerated and resulted in only mild and transient procedure- or drug-related adverse events [93, 94]. Transient subclinical induction of inflammatory markers has also been reported [87, 95]. Despite the congenital deprivation of cone-driven light signaling in patients with *CNGA3*-ACHM, treatment with AAV8.CNGA3 resulted in improvements in secondary endpoints related to cone function, including increases in visual acuity and contrast sensitivity against baseline in all nine treated patients [93], which persisted until at least 3 years after treatment [94]. A phase IIb clinical trial targeting treatment of the second eye of the first patients and treatment of children aged 6–12 years is ongoing (Table 1). Four other programs are currently in phase I/II of clinical trials, two on *CNGA3*-ACHM and two additional programs on *CNGB3*-ACHM (Table 1), but their data have not yet been published.

7 Conclusions and Outlook

In recent years, gene therapy has emerged as a viable treatment option for ACHM. Promising rAAV-based gene transfer technologies have been evaluated for safe and efficient gene delivery to the cone photoreceptors in multiple species. Several promising rAAV-based treatments for the most common forms of ACHM caused by mutations in *CNGA3* or *CNGB3* are currently being investigated in clinical trials. Although AAVs have natural limitations as a gene delivery system, vector engineering may help to develop improved rAAV variants that support transduction of a greater proportion of cone photoreceptors with higher efficiency and lower immunogenicity, ideally via less invasive administration routes.

Declarations

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Author contributions SM wrote the manuscript; all other authors contributed to the writing of the manuscript.

Ethics approval Not applicable.

Consent to participate Not applicable.

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References

1. Francois J. Heredity in ophthalmology. Bull Soc Belge Ophtalmol. 1958;118(Pt 1):1–300.
2. Neitz J, Neitz M. The genetics of normal and defective color vision. Vis Res. 2011;51(7):633–51.
3. Hirji N, Aboshiha J, Georgiou M, Bainbridge J, Michaelides M. Achromatopsia: clinical features, molecular genetics, animal models and therapeutic options. Ophthalmic Genet. 2018;39(2):149–57.
4. Brunetti-Pierri R, Karali M, Melillo P, Di Iorio V, De Benedictis A, Iaccarino G, et al. Clinical and molecular characterization of achromatopsia patients: a longitudinal study. Int J Mol Sci. 2021;7(22):4.
5. Andreasson S, Tornqvist K. Electroretinograms in patients with achromatopsia. Acta Ophthalmol (Copenh). 1991;69(6):711–6.
6. Felden J, Baumann B, Ali M, Audo I, Ayuso C, Bocquet B, et al. Mutation spectrum and clinical investigation of achromatopsia patients with mutations in the GNAT2 gene. Hum Mutat. 2019;40(8):1145–55.
7. Kohl S, Varsanyi B, Antunes GA, Baumann B, Hoyng CB, Jagle H, et al. CNGB3 mutations account for 50% of all cases with autosomal recessive achromatopsia. Eur J Hum Genet. 2005;13(3):302–8.

8. Mayer AK, Van Cauwenbergh C, Rother C, Baumann B, Reuter P, De Baere E, et al. CNGB3 mutation spectrum including copy number variations in 552 achromatopsia patients. Hum Mutat. 2017;38(11):1579–91.
9. Michalakis S, Schön C, Becirovic E, Biel M. Gene therapy for achromatopsia. J Gene Med. 2017;19:3.
10. Weisschuh N, Obermaier CD, Battke F, Bernd A, Kuehlewein L, Nasser F, et al. Genetic architecture of inherited retinal degeneration in Germany: a large cohort study from a single diagnostic center over a 9-year period. Hum Mutat. 2020;41(9):1514–27.
11. Georgiou M, Fujinami K, Michaelides M. Inherited retinal diseases: therapeutics, clinical trials and end points—a review. Clin Exp Ophthalmol. 2021;49(3):270–88.
12. Kohl S, Zobor D, Chiang WC, Weisschuh N, Staller J, Gonzalez Menendez I, et al. Mutations in the unfolded protein response regulator ATF6 cause the cone dysfunction disorder achromatopsia. Nat Genet. 2015;47(7):757–65.
13. Michalakis S, Becirovic E, Biel M. Retinal cyclic nucleotide-gated channels: from pathophysiology to therapy. Int J Mol Sci. 2018;7(19):3.
14. Yamamoto K, Sato T, Matsui T, Sato M, Okada T, Yoshida H, et al. Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. Dev Cell. 2007;13(3):365–76.
15. Haze K, Yoshida H, Yanagi H, Yura T, Mori K. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. Mol Biol Cell. 1999;10(11):3787–99.
16. Zhong H, Molday LL, Molday RS, Yau KW. The heteromeric cyclic nucleotide-gated channel adopts a 3A:1B stoichiometry. Nature. 2002;420(6912):193–8.
17. Lamb TD. Evolution of the genes mediating phototransduction in rod and cone photoreceptors. Prog Retin Eye Res. 2020;76:100823.
18. Luo DG, Xue T, Yau KW. How vision begins: an odyssey. Proc Natl Acad Sci USA. 2008;105(29):9855–62.
19. Hanany M, Rivolta C, Sharon D. Worldwide carrier frequency and genetic prevalence of autosomal recessive inherited retinal diseases. Proc Natl Acad Sci USA. 2020;117(5):2710–6.
20. Remmer MH, Rastogi N, Ranka MP, Ceisler EJ. Achromatopsia: a review. Curr Opin Ophthalmol. 2015;26(5):333–40.
21. Burkard M, Kohl S, Kratzig T, Tanimoto N, Brennenstuhl C, Bausch AE, et al. Accessory heterozygous mutations in cone photoreceptor CNGA3 exacerbate CNG channel-associated retinopathy. J Clin Investig. 2018;128(12):5663–75.
22. Sundin OH, Yang JM, Li Y, Zhu D, Hurd JN, Mitchell TN, et al. Genetic basis of total colourblindness among the Pingelapese islanders. Nat Genet. 2000;25(3):289–93.
23. Sheffield VC. The vision of typhoon lengkieki. Nat Med. 2000;6(7):746–7.
24. Zelinger L, Cideciyan AV, Kohl S, Schwartz SB, Rosenmann A, Eli D, et al. Genetics and disease expression in the CNGA3 form of achromatopsia: steps on the path to gene therapy. Ophthalmology. 2015;122(5):997–1007.
25. Liang X, Dong F, Li H, Li H, Yang L, Sui R. Novel CNGA3 mutations in Chinese patients with achromatopsia. Br J Ophthalmol. 2015;99(4):571–6.
26. Sun W, Li S, Xiao X, Wang P, Zhang Q. Genotypes and phenotypes of genes associated with achromatopsia: a reference for clinical genetic testing. Mol Vis. 2020;26:588–602.
27. Aboshiha J, Dubis AM, Carroll J, Hardcastle AJ, Michaelides M. The cone dysfunction syndromes. Br J Ophthalmol. 2016;100(1):115–21.
28. Poloschek CM, Kohl S. Achromatopsia. Ophthalmologe. 2010;107(6):571–80.
29. Tager J, Wissinger B, Kohl S, Reuter P. Identification of chemical and pharmacological chaperones for correction of trafficking-deficient mutant cyclic nucleotide-gated A3 channels. Mol Pharmacol. 2021;99(6):460–8.
30. Brennenstuhl C, Tanimoto N, Burkard M, Wagner R, Bolz S, Trifunovic D, et al. Targeted ablation of the Pde6h gene in mice reveals cross-species differences in cone and rod phototransduction protein isoform inventory. J Biol Chem. 2015;290(16):10242–55.
31. Buena-Atienza E, Ruther K, Baumann B, Bergholz R, Birch D, De Baere E, et al. De novo intrachromosomal gene conversion from OPN1MW to OPN1LW in the male germline results in Blue Cone Monochromacy. Sci Rep. 2016;24(6):28253.
32. Dai G, Varnum MD. CNGA3 achromatopsia-associated mutation potentiates the phosphoinositide sensitivity of cone photoreceptor CNG channels by altering intersubunit interactions. Am J Physiol Cell Physiol. 2013;305(2):C147–59.
33. Duricka DL, Brown RL, Varnum MD. Defective trafficking of cone photoreceptor CNG channels induces the unfolded protein response and ER-stress-associated cell death. Biochem J. 2012;441(2):685–96.
34. Koeppen K, Reuter P, Kohl S, Baumann B, Ladewig T, Wissinger B. Functional analysis of human CNGA3 mutations associated with colour blindness suggests impaired surface expression of channel mutants A3(R427C) and A3(R563C). Eur J Neurosci. 2008;27(9):2391–401.
35. Koeppen K, Reuter P, Ladewig T, Kohl S, Baumann B, Jacobson SG, et al. Dissecting the pathogenic mechanisms of mutations in the pore region of the human cone photoreceptor cyclic nucleotide-gated channel. Hum Mutat. 2010;31(7):830–9.
36. Kuniyoshi K, Muraki-Oda S, Ueyama H, Toyoda F, Sakuramoto H, Ogita H, et al. Novel mutations in the gene for alpha-subunit of retinal cone cyclic nucleotide-gated channels in a Japanese patient with congenital achromatopsia. Jpn J Ophthalmol. 2016;60(3):187–97.
37. Liu C, Varnum MD. Functional consequences of progressive cone dystrophy-associated mutations in the human cone photoreceptor cyclic nucleotide-gated channel CNGA3 subunit. Am J Physiol Cell Physiol. 2005;289(1):C187–98.
38. Matveev AV, Fitzgerald JB, Xu J, Malykhina AP, Rodgers KK, Ding XQ. The disease-causing mutations in the carboxyl terminus of the cone cyclic nucleotide-gated channel CNGA3 subunit alter the local secondary structure and interfere with the channel active conformational change. Biochemistry. 2010;49(8):1628–39.
39. Meighan PC, Peng C, Varnum MD. Inherited macular degeneration-associated mutations in CNGB3 increase the ligand sensitivity and spontaneous open probability of cone cyclic nucleotide-gated channels. Front Physiol. 2015;6:177.
40. Muraki-Oda S, Toyoda F, Okada A, Tanabe S, Yamade S, Ueyama H, et al. Functional analysis of rod monochromacy-associated missense mutations in the CNGA3 subunit of the cone photoreceptor cGMP-gated channel. Biochem Biophys Res Commun. 2007;362(1):88–93.
41. Patel KA, Bartoli KM, Fandino RA, Ngatchou AN, Woch G, Carey J, et al. Transmembrane S1 mutations in CNGA3 from achromatopsia 2 patients cause loss of function and impaired cellular trafficking of the cone CNG channel. Invest Ophthalmol Vis Sci. 2005;46(7):2282–90.
42. Reuter P, Koeppen K, Ladewig T, Kohl S, Baumann B, Wissinger B, et al. Mutations in CNGA3 impair trafficking or function of cone cyclic nucleotide-gated channels, resulting in achromatopsia. Hum Mutat. 2008;29(10):1228–36.
43. Shaikh RS, Reuter P, Sisk RA, Kausar T, Shahzad M, Maqsood MI, et al. Homozygous missense variant in the human CNGA3

channel causes cone-rod dystrophy. Eur J Hum Genet. 2015;23(4):473–80.
44. Täger J, Kohl S, Birch DG, Wheaton DKH, Wissinger B, Reuter P. An early nonsense mutation facilitates the expression of a short isoform of CNGA3 by alternative translation initiation. Exp Eye Res. 2018;171:48–53.
45. Täger J, Wissinger B, Kohl S, Reuter P. Identification of chemical and pharmacological chaperones for correction of trafficking-deficient mutant cyclic nucleotide-gated A3 channels. Mol Pharmacol. 2021;99(6):460–8.
46. Tränkner D, Jägle H, Kohl S, Apfelstedt-Sylla E, Sharpe LT, Kaupp UB, et al. Molecular basis of an inherited form of incomplete achromatopsia. J Neurosci. 2004;24(1):138–47.
47. Sundaram V, Wilde C, Aboshiha J, Cowing J, Han C, Langlo CS, et al. Retinal structure and function in achromatopsia: implications for gene therapy. Ophthalmology. 2014;121(1):234–45.
48. Michalakis S, Geiger H, Haverkamp S, Hofmann F, Gerstner A, Biel M. Impaired opsin targeting and cone photoreceptor migration in the retina of mice lacking the cyclic nucleotide-gated channel CNGA3. Invest Ophthalmol Vis Sci. 2005;46(4):1516–24.
49. Arango-Gonzalez B, Trifunovic D, Sahaboglu A, Kranz K, Michalakis S, Farinelli P, et al. Identification of a common non-apoptotic cell death mechanism in hereditary retinal degeneration. PLoS ONE. 2014;9(11):e112142.
50. Koch M, Scheel C, Ma H, Yang F, Stadlmeier M, Gluck AF, et al. The cGMP-dependent protein kinase 2 contributes to cone photoreceptor degeneration in the Cnga3-deficient mouse model of achromatopsia. Int J Mol Sci. 2020;22:1.
51. Lin Q, Lv JN, Wu KC, Zhang CJ, Liu Q, Jin ZB. Generation of nonhuman primate model of cone dysfunction through in situ AAV-mediated CNGB3 ablation. Mol Ther Methods Clin Dev. 2020;11(18):869–79.
52. Thapa A, Morris L, Xu J, Ma H, Michalakis S, Biel M, et al. Endoplasmic reticulum stress-associated cone photoreceptor degeneration in cyclic nucleotide-gated channel deficiency. J Biol Chem. 2012;287(22):18018–29.
53. Xu J, Morris L, Thapa A, Ma H, Michalakis S, Biel M, et al. cGMP accumulation causes photoreceptor degeneration in CNG channel deficiency: evidence of cGMP cytotoxicity independently of enhanced CNG channel function. J Neurosci. 2013;33(37):14939–48.
54. Thomas MG, Kumar A, Kohl S, Proudlock FA, Gottlob I. High-resolution in vivo imaging in achromatopsia. Ophthalmology. 2011;118(5):882–7.
55. Langlo CS, Patterson EJ, Higgins BP, Summerfelt P, Razeen MM, Erker LR, et al. Residual foveal cone structure in CNGB3-associated achromatopsia. Invest Ophthalmol Vis Sci. 2016;57(10):3984–95.
56. Dubis AM, Cooper RF, Aboshiha J, Langlo CS, Sundaram V, Liu B, et al. Genotype-dependent variability in residual cone structure in achromatopsia: toward developing metrics for assessing cone health. Invest Ophthalmol Vis Sci. 2014;55(11):7303–11.
57. Moshiri A, Chen R, Kim S, Harris RA, Li Y, Raveendran M, et al. A nonhuman primate model of inherited retinal disease. J Clin Investig. 2019;129(2):863–74.
58. Reicher S, Seroussi E, Gootwine E. A mutation in gene CNGA3 is associated with day blindness in sheep. Genomics. 2010;95(2):101–4.
59. Tanaka N, Dutrow EV, Miyadera K, Delemotte L, MacDermaid CM, Reinstein SL, et al. Canine CNGA3 gene mutations provide novel insights into human achromatopsia-associated channelopathies and treatment. PLoS ONE. 2015;10(9):e0138943.
60. Sidjanin DJ, Lowe JK, McElwee JL, Milne BS, Phippen TM, Sargan DR, et al. Canine CNGB3 mutations establish cone degeneration as orthologous to the human achromatopsia locus ACHM3. Hum Mol Genet. 2002;11(16):1823–33.
61. Yeh CY, Goldstein O, Kukekova AV, Holley D, Knollinger AM, Huson HJ, et al. Genomic deletion of CNGB3 is identical by descent in multiple canine breeds and causes achromatopsia. BMC Genet. 2013;20(14):27.
62. Pang JJ, Deng WT, Dai X, Lei B, Everhart D, Umino Y, et al. AAV-mediated cone rescue in a naturally occurring mouse model of CNGA3-achromatopsia. PLoS ONE. 2012;7(4):e35250.
63. Shamir MH, Ofri R, Bor A, Brenner O, Reicher S, Obolensky A, et al. A novel day blindness in sheep: epidemiological, behavioural, electrophysiological and histopathological studies. Vet J. 2010;185(2):130–7.
64. Ding XQ, Harry CS, Umino Y, Matveev AV, Fliesler SJ, Barlow RB. Impaired cone function and cone degeneration resulting from CNGB3 deficiency: down-regulation of CNGA3 biosynthesis as a potential mechanism. Hum Mol Genet. 2009;18(24):4770–80.
65. Hassall MM, Barnard AR, Orlans HO, McClements ME, Charbel Issa P, Aslam SA, et al. A novel achromatopsia mouse model resulting from a naturally occurring missense change in Cngb3. Invest Ophthalmol Vis Sci. 2018;59(15):6102–10.
66. Biel M, Seeliger M, Pfeifer A, Kohler K, Gerstner A, Ludwig A, et al. Selective loss of cone function in mice lacking the cyclic nucleotide-gated channel CNG3. Proc Natl Acad Sci USA. 1999;96(13):7553–7.
67. Michalakis S, Mühlfriedel R, Tanimoto N, Krishnamoorthy V, Koch S, Fischer MD, et al. Restoration of cone vision in the CNGA3-/- mouse model of congenital complete lack of cone photoreceptor function. Mol Therapy J Am Soc Gene Therapy. 2010;18(12):2057–63.
68. Michalakis S, Xu J, Biel M, Ding XQ. Detection of cGMP in the degenerating retina. Methods Mol Biol. 2013;1020:235–45.
69. Zhang T, Zhang N, Baehr W, Fu Y. Cone opsin determines the time course of cone photoreceptor degeneration in Leber congenital amaurosis. Proc Natl Acad Sci USA. 2011;108(21):8879–84.
70. Dixon CJ. Achromatopsia in three sibling Labrador Retrievers in the UK. Vet Ophthalmol. 2016;19(1):68–72.
71. Chang B, Dacey MS, Hawes NL, Hitchcock PF, Milam AH, Atmaca-Sonmez P, et al. Cone photoreceptor function loss-3, a novel mouse model of achromatopsia due to a mutation in Gnat2. Invest Ophthalmol Vis Sci. 2006;47(11):5017–21.
72. Chang B, Grau T, Dangel S, Hurd R, Jurklies B, Sener EC, et al. A homologous genetic basis of the murine cpfl1 mutant and human achromatopsia linked to mutations in the PDE6C gene. Proc Natl Acad Sci USA. 2009;106(46):19581–6.
73. Kohl S, Hamel C. Clinical utility gene card for: Achromatopsia—update 2013. Eur J Hum Genet. 2013;21:11.
74. Auricchio A, Smith AJ, Ali RR. The future looks brighter after 25 years of retinal gene therapy. Hum Gene Ther. 2017;28(11):982–7.
75. Carvalho LS, Xu J, Pearson RA, Smith AJ, Bainbridge JW, Morris LM, et al. Long-term and age-dependent restoration of visual function in a mouse model of CNGB3-associated achromatopsia following gene therapy. Hum Mol Genet. 2011;20(16):3161–75.
76. Komaromy AM, Alexander JJ, Rowlan JS, Garcia MM, Chiodo VA, Kaya A, et al. Gene therapy rescues cone function in congenital achromatopsia. Hum Mol Genet. 2010;19(13):2581–93.
77. Michalakis S, Muhlfriedel R, Tanimoto N, Krishnamoorthy V, Koch S, Fischer MD, et al. Restoration of cone vision in the CNGA3-/- mouse model of congenital complete lack of cone photoreceptor function. Mol Therapy J Am Soc Gene Therapy. 2010;18(12):2057–63.
78. Alexander JJ, Umino Y, Everhart D, Chang B, Min SH, Li Q, et al. Restoration of cone vision in a mouse model of achromatopsia. Nat Med. 2007;13(6):685–7.
79. Mühlfriedel R, Tanimoto N, Schon C, Sothilingam V, Garcia Garrido M, Beck SC, et al. AAV-mediated gene supplementation

therapy in achromatopsia type 2: preclinical data on therapeutic time window and long-term effects. Front Neurosci. 2017;11:292.
80. Dai X, He Y, Zhang H, Zhang Y, Liu Y, Wang M, et al. Long-term retinal cone rescue using a capsid mutant AAV8 vector in a mouse model of CNGA3-achromatopsia. PLoS ONE. 2017;12(11):e0188032.
81. Du W, Tao Y, Deng WT, Zhu P, Li J, Dai X, et al. Vitreal delivery of AAV vectored Cnga3 restores cone function in CNGA3-/-/Nrl-/- mice, an all-cone model of CNGA3 achromatopsia. Hum Mol Genet. 2015;24(13):3699–707.
82. Pavlou M, Schön C, Occelli LM, Rossi A, Meumann N, Boyd RF, et al. Novel AAV capsids for intravitreal gene therapy of photoreceptor disorders. EMBO Mol Med. 2021;2021:e13392.
83. Banin E, Gootwine E, Obolensky A, Ezra-Elia R, Ejzenberg A, Zelinger L, et al. Gene augmentation therapy restores retinal function and visual behavior in a sheep model of CNGA3 achromatopsia. Mol Therapy J Am Soc Gene Therapy. 2015;23(9):1423–33.
84. Gootwine E, Ofri R, Banin E, Obolensky A, Averbukh E, Ezra-Elia R, et al. Safety and efficacy evaluation of rAAV2tYF-PR1.7-hCNGA3 vector delivered by subretinal injection in CNGA3 mutant achromatopsia sheep. Hum Gene Ther Clin Dev. 2017;28(2):96–107.
85. Ofri R, Averbukh E, Ezra-Elia R, Ross M, Honig H, Obolensky A, et al. Six years and counting: restoration of photopic retinal function and visual behavior following gene augmentation therapy in a sheep model of CNGA3 achromatopsia. Hum Gene Ther. 2018;29(12):1376–86.
86. Tobias P, Philipp SI, Stylianos M, Martin B, Barbara W, Felix R, et al. Safety and toxicology of ocular gene therapy with recombinant AAV vector rAAVh.CNGA3 in nonhuman primates. Hum Gene Ther Clin Dev. 2019;30(2):50–6.
87. Reichel FF, Peters T, Wilhelm B, Biel M, Ueffing M, Wissinger B, et al. Humoral immune response after intravitreal but not after subretinal AAV8 in primates and patients. Invest Ophthalmol Vis Sci. 2018;59(5):1910–5.
88. Seitz IP, Michalakis S, Wilhelm B, Reichel FF, Ochakovski GA, Zrenner E, et al. Superior retinal gene transfer and biodistribution profile of subretinal versus intravitreal delivery of AAV8 in nonhuman primates. Invest Ophthalmol Vis Sci. 2017;58(13):5792–801.
89. Komaromy AM, Rowlan JS, Corr AT, Reinstein SL, Boye SL, Cooper AE, et al. Transient photoreceptor deconstruction by CNTF enhances rAAV-mediated cone functional rescue in late stage CNGB3-achromatopsia. Mol Therapy J Am Soc Gene Therapy. 2013;21(6):1131–41.
90. Ye GJ, Budzynski E, Sonnentag P, Nork TM, Miller PE, McPherson L, et al. Safety and biodistribution evaluation in CNGB3-deficient mice of rAAV2tYF-PR1.7-hCNGB3, a recombinant AAV vector for treatment of achromatopsia. Hum Gene Ther Clin Dev. 2016;27(1):27–36.
91. Ye GJ, Budzynski E, Sonnentag P, Nork TM, Miller PE, Sharma AK, et al. Safety and biodistribution evaluation in cynomolgus macaques of rAAV2tYF-PR1.7-hCNGB3, a recombinant AAV Vector for treatment of achromatopsia. Hum Gene Ther Clin Dev. 2016;27(1):37–48.
92. Ye GJ, Komaromy AM, Zeiss C, Calcedo R, Harman CD, Koehl KL, et al. Safety and efficacy of AAV5 vectors expressing human or canine CNGB3 in CNGB3-mutant dogs. Hum Gene Ther Clin Dev. 2017;28(4):197–207.
93. Fischer MD, Michalakis S, Wilhelm B, Zobor D, Muehlfriedel R, Kohl S, et al. Safety and vision outcomes of subretinal gene therapy targeting cone photoreceptors in achromatopsia: a nonrandomized controlled trial. JAMA Ophthalmol. 2020;138(6):643–51.
94. Reichel FF, Michalakis S, Wilhelm B, Zobor D, Muehlfriedel R, Kohl S, et al. Three-year results of phase I retinal gene therapy trial for CNGA3-mutated achromatopsia: results of a non randomised controlled trial. Br J Ophthalmol. 2021. https://doi.org/10.1136/bjophthalmol-2021-319067.
95. Reichel FF, Dauletbekov DL, Klein R, Peters T, Ochakovski GA, Seitz IP, et al. AAV8 Can induce innate and adaptive immune response in the primate eye. Mol Therapy J Am Soc Gene Therapy. 2017;25(12):2648–2660.
96. Mühlfriedel R, Tanimoto N, Schön C, Sothilingam V, Garcia Garrido M, Beck SC, et al. AAV-mediated gene supplementation therapy in achromatopsia type 2: preclinical data on therapeutic time window and long-term effects. Front Neurosci. 2017;11:292.
97. Garcia MM, Ying GS, Cocores CA, Tanaka JC, Komaromy AM. Evaluation of a behavioral method for objective vision testing and identification of achromatopsia in dogs. Am J Vet Res. 2010;71(1):97–102.