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 \textit{CNGA3} or \textit{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 \textit{CNGA3}- and \textit{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.
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+/Ca2+ 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 Ca2+ 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 have unique properties.

| 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], cpf15 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   | Cpf1 mouse [71] | [78]           |
| PDE6C           | 10q23.3,33      | Cone dystrophy 4/600827 | Cpf1 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

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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 cone photoreceptors unable to respond to light [66], accompanied by progressive degeneration 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 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 Cpfβ3 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 GNA12-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 cilurotrophic 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 | MeiraGTx/Janssen         | 03001310   | Completed recruiting |
| CNGB3    | AAV8-hCAR-hCNGB3     | I/II  | RD-CURE                 | 02610582   | Completed recruiting |
| CNGB3    | AAV8-hG1.7-hCNGB3    | I/II  | MeiraGTx/Janssen         | 03758404   | Completed recruiting |
| CNGB3    | AAV2tYF-PR1.7-hCNGB3 | I/II  | AGTC                    | 02935517   | Recruiting     |

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

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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 × 1010 to 1 × 1011 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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