Gene Therapy for Achromatopsia

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Abstract

Aim This review summarizes the current status of Achromatopsia (ACHM) gene therapy-related research activities and provides an outlook for their clinical application.

Discussion ACHM is an inherited eye disease characterized by congenital absence of cone photoreceptor function. As a consequence, ACHM is associated with strongly impaired

daylight vision, photophobia, nystagmus, and lack of color discrimination. Currently, six genes have been linked to ACHM. Up to 80% of the patients carry mutations in the genes *CNGA3* and *CNGB3* encoding the two subunits of the cone cyclic nucleotide-gated (CNG) channel. Various animal models of the disease have been established and their characterization helped to increase our understanding of the pathophysiology associated with ACHM. With the advent of adeno-associated virus (AAV) vectors as valuable gene delivery tools for retinal photoreceptors a number of promising gene supplementation therapy programs have been initiated.

Conclusion During the last years, huge progress has been made towards bringing a curative treatment for ACHM into clinics. First clinical trials are ongoing or will be launched soon and are expected to contribute important data on the safety and efficacy of ACHM gene supplementation therapy.

Clinical manifestation, etiology and genetics of Achromatopsia

Achromatopsia (ACHM) is an autosomal recessive eye disorder with an overall prevalence of approximately 1:30,000 [1-3]. ACHM patients suffer from severely impaired daylight vision, poor visual acuity, photophobia, nystagmus (involuntary rapid eye movements), and lack of the ability to discriminate colors [4] (Figure 1). In addition to these functional defects, ACHM patients present with varying degrees of cone photoreceptor degeneration. Mutations in six genes (*ATF6, CNGA3, CNGB3, GNAT2, PDE6C* and *PDE6H*) are currently implicated in ACHM (Table 1). Up to 80% of ACHM patients carry mutations in *CNGA3* or *CNGB3*. These genes encode the two subunits of the cyclic nucleotide-gated (CNG) channel expressed in cone photoreceptor outer segments [4, 5]. The cone CNG channel is a heterotetrameric complex assembled by three CNGA3 and one CNGB3 subunits [6]. Functionally, CNG channels are an essential component of the phototransduction cascade that mediates the

translation of light-triggered changes in the second messenger cyclic guanosine monophosphate (cGMP) into a voltage and calcium signal [4]. In the dark, binding of cGMP to the CNG channels leads to opening of the channels and depolarization of the cone photoreceptor cell. Following light stimulation cGMP is hydrolyzed by cone phosphodiesterase (PDE) activity, the CNG channels close and the cone becomes hyperpolarized. This hyperpolarization reduces the release of the neurotransmitter glutamate at the synaptic terminal and triggers downstream visual signaling.

To date, more than 100 mutations in *CNGA3* and more than 50 mutations in *CNGB3* were found to cause inherited ACHM in humans [7]. *CNGB3* mutations are more prevalent (estimated fractional prevalence: 50 %) in Europe and the United States, whereas *CNGA3* is the most frequent disease gene in the Middle East and China (estimated fractional prevalence: 60 %). Most *CNGB3* mutations are nonsense, frameshift or splice mutations resulting in truncated or strongly impaired channel proteins [2, 5]. However, the majority of *CNGA3* mutations are missense mutations affecting only single amino acid residues of the protein [2, 5]. All other known ACHM genes, except *ATF6*, also encode for essential components of the cone phototransduction cascade, e.g. subunits of the cone PDE complex (*PDE6C* and *PDE6H*) or the guanine nucleotide-binding protein (G protein), (*GNAT2*) which signals between rhodopsin and the PDE. Absence or malfunction of any of those phototransduction genes results in functional impairment of the signaling cascade. However, the exact mechanisms linking mutations in these phototransduction genes to the ACHM pathology have not been established yet. The latest addition to the ACHM genes is *ATF6*, which encodes for a transcription factor involved in the unfolded protein response. It was suggested that an increased susceptibility to ER stress-induced damage during retinal development underlies the pathology of ACHM in patients with *ATF6* mutations [8].

In summary, ACHM is genetically well characterized. The majority of cases are linked to CNG channel gene mutations. Mutations in the four non-CNG channel encoding ACHM genes are much less frequent and together account for less than 6 % of the cases. Not yet identified putative ACHM genes could account for approximately 5 % of the cases.

Animal models of Achromatopsia

Several engineered and naturally occurring small and large animal models of ACHM exist (Table 1). The first described animal model was the *Cnga3* knockout (KO) mouse, which helped establishing the molecular basis of *CNGA3*-linked ACHM (ACHM2, OMIM #21690) [9]. Genetic inactivation of *Cnga3* in mice leads to selective loss of cone-mediated light responses [9] accompanied by progressive degeneration and cell death of cones [10]. In addition, a naturally occurring mouse model of ACHM2 - the *cpfl*5 mouse with a *Cnga3* point mutation - was described with a phenotype similar to the *Cnga3* KO mouse [11]. Moreover, a sheep model of ACHM2 was identified with diminished cone, but normal rod function [12, 13]. Affected lambs were homozygous for a mutation in the *Cnga3* gene that results in substitution of amino acid R236 by a stop codon [12]. Recently, two spontaneous canine models of ACHM2 have been described [14]: a German shepherd, carrying a CNGA3/p.R424W mutation and a Labrador retriever with a CNGA3/p.V644del mutation.

Small and large animal models also exist for CNGB3-associated ACHM (ACHM3, OMIM #262300). Knockout of *Cngb3* in mice results in strongly reduced, but not absent cone function and progressive cone photoreceptor degeneration [15, 16]. The residual cone function in this model can be attributed to irregular homomeric CNGA3 channels transported to the outer segments in absence of CNGB3 protein [17]. In addition to the *Cngb3* knockout (KO) mouse, several spontaneous dog models exist that carry recessive mutations in exon 6

or a genomic deletion of the entire CNGB3 gene and present with a clinical phenotype of day-blindness [18-20]. Mouse models also exist for the other less frequent ACHM genes [21-24].

Development of gene supplementation therapy

Clinical management of ACHM is currently limited to specialized genetic counseling, the use of low vision aids, tinted contact lenses or glasses to reduce symptoms of photophobia [3, 25]. However, no curative treatment for ACHM exists. The availability of suitable animal models and recombinant adeno-associated virus (rAAV) vectors as efficient and safe retinal gene transfer vectors allowed for the preclinical development of novel ACHM gene therapies. As described above, ACHM is caused by missense or loss-of-function mutations that are inherited in an autosomal recessive manner. Therefore, this disease is a very attractive candidate for so-called gene supplementation approaches. Gene supplementation aims at complementing affected cone photoreceptor cells with a healthy copy of the disease-causing gene. In the following paragraphs, we will provide an overview on rAAV vectors, review current preclinical activities and finally summarize ongoing clinical trials.

rAAV vectors as gene therapy tools

AAVs are small (25 nm diameter), non-enveloped and non-pathogenic viruses that belong to the *Parvovirus* family and *Dependovirus* genus and accordingly depend on the presence of adeno-, papilloma- or herpes-viruses for replication [26]. The wildtype AAV genome consists of approx. 4.7 kb single stranded DNA with two open reading frames (*rep* and *cap*) flanked by two inverted terminal repeats (ITRs) [26]. ITRs are 145 bp palindromic sequences forming

hairpin-loops and are critical components required for proper AAV genome packaging, transcription and other essential functions [27, 28]. There are several properties that render AAVs perfectly suited as neuronal (and in particular retinal) gene transfer vectors: (1) Their small genome can be easily manipulated and recombinant AAVs are easy to produce and purify. (2) The only AAV genomic sequences that have to be present in a typical AAV expression (*cis*) plasmid are the two "flanking" ITRs [29, 30], (3) Thus, the viral reading frames *rep* and *cap* can be excised from the AAV genome and provided in *trans* during the rAAV production process using helper plasmids (or helper virus e.g. adenovirus). Thereby, approx. 4.5 kb cargo capacity between the ITRs becomes available for a gene expression cassette. (4) Helper virus-free protocols have been established for the production of rAAV particles in eukaryotic cells or in insect cells [31, 32]. In these protocols, the *cis* plasmid with the desired gene expression cassette, the *rep* and *cap* expressing plasmids and the helper plasmid containing the adenoviral helper genes E1A, E1B, E2A, E4, and VARNA are co-transfected into producer cells (e.g. HEK293 cells) [32, 33]. rAAVs can then be isolated from the cell culture supernatant or the lysed cells and purified using standard gradient centrifugation, chromatography and size exclusion techniques [32, 33]. (5) Various naturally occurring or engineered AAV capsids are available allowing for packaging of pseudotyped rAAV of different serotypes with distinct properties, e.g. specificity for different cell types [34, 35]. (6) Finally, rAAVs are considered as very save gene therapy vectors with low immunogenicity and toxicity [28, 36]. After transduction, the rAAV vector genome stays predominantly in an episomal state. Although studies suggest that rAAV vectors can integrate into the host genome, no major insertional mutagenesis issues have been reported [37-39].

Gene supplementation therapy: preclinical studies

Successful proof-of-concept animal studies for rAAV-based gene supplementation therapy exist for *CNGA3*, *CNGB3* and *GNAT2* [11, 40-43]. In the past, we have utilized the well characterized *Cnga3* KO mouse model to evaluate rAAV-mediated *Cnga3* gene supplementation as a potential treatment of *CNGA3*-linked ACHM [42]. In this approach, we subretinally applied AAV5-Y719F-pseudotyped vector particles that expressed the mouse *Cnga3* cDNA under control of a mouse S-opsin promoter [42]. We showed that such gene supplementation was able to restore cone-specific visual processing in the central nervous system of *Cnga3* KO mice. Treated *Cnga3* KO retinas were able to generate cone-driven light responses. The treatment had also beneficial effects on retinal morphology and degenerative cellular processes. For instance, the therapy normalized pathologically elevated cellular cGMP levels in cones, delayed cone cell death and diminished the inflammatory response of Müller glia cells that is typical for retinal degenerations [10]. Follow-up studies suggest that the therapeutic effect was long-lasting (e.g. stable for at least 8 months after treatment) [44] and could be applied at more advanced disease stages (e.g. at 1 or 3 months of age) [44]. Moreover, a positive therapeutic outcome was also obtained using rAAV vectors pseudotyped with other capsids (e.g. AAV8) and alternative cone-specific promoters (e.g. a human red/green opsin promoter, cone arrestin) [44]. A similar *Cnga3* gene supplementation approach was described by another group using the *cpfl5* mouse, a naturally occurring *Cnga3* mutant mouse model [11]. This approach was based on an AAV5-pseudotyped vector with a gene expression cassette containing the murine *Cnga3* cDNA under control of the ubiquitous chicken β-actin promoter. Using this vector, a substantial restoration of cone-mediated ERG, normalization of visual acuity and contrast sensitivity, and arrest of cone degeneration was described. The therapeutic effect was observed for at least 5-months post-injection [11]. More recently, a successful gene supplementation was also described for the *Cnga3* mutant sheep

model [45]. The approach utilized AAV5-peusodtyped vectors expressing either mouse *Cnga3* or human *CNGA3* under control of a 2.1 kb human red cone opsin promoter. An impressive restoration of cone function (ERG) and daylight vision was accomplished which was maintained up to 3 years after treatment.

Successful rAAV-based gene supplementation was also achieved in animal models of *CNGB3*-related ACHM [40, 41]. Subretinal delivery of AAV5-peusodtyped vectors expressing human *CNGB3* under control of a 2.1 kb human red cone opsin promoter led to long-term restoration of cone function and daylight vision in two canine models of CNGB3-related ACHM [41]. Interestingly, the treatment was less efficient if treated dogs were already at a more advanced stage of disease [41]. To circumvent this phenomenon, the same group combined the gene therapeutic approach with the administration of ciliary neurotrophic factor (CNTF) [46]. CNTF is known to cause a temporal deconstruction of photoreceptor outer segments. This reversible effect leads to immature photoreceptors with a temporal shortening of outer segments and reduction of gene expression. In combination with such CNTF-mediated deconstruction and regeneration the gene therapy was also successful in advanced stage *Cngb3* dogs (14 to 42-month-old).

Another approach tested the efficacy of a *CNGB3* gene supplementation therapy in *Cngb3* KO mice [40]. This approach was based on an AAV8-pseudotyped vector containing a human cone arrestin promoter driving the expression of human *CNGB3*. When delivered subretinally, this vector led to reconstitution of heterotetrameric cone CNG channels, improvement of retinal morphology and prolonged survival of cones. Moreover, the therapy resulted in a long-term improvement of retinal function in *Cngb3* KO mice, which was shown by the restoration of cone ERG amplitudes and improvement in visual acuity. Interestingly,

successful restoration of cone function was also observed when the treatment was initiated at 6 months of age. However, restoration of normal visual acuity was only possible when treatment was applied to 2-4 weeks old animals [40].

A successful gene supplementation therapy was also described for *GNAT2*-linked ACHM [43]. In this study, the naturally occurring *Cpfl3* mouse line was used, a hypomorph *Gnat2*-mutant with residual protein expression and impaired cone ERG responses (approx. 25 % of normal level at 4 weeks of age), but no obvious cone degeneration. In this approach, an AAV5-pseudotyped vector carrying the mouse *Gnat2* cDNA driven by the 2.1 kb human red cone opsin promoter was delivered into the subretinal space of *Cpfl3* mice of various ages. A clear positive effect of the gene supplementation could be observed at the level of cone ERG and optomotor behavior for at least 7 months after treatment [43].

Clinical trials with Achromatopsia patients

ACHM has previously been considered as a stationary disease: The cone ERG is either missing from birth or the functional loss does not progress with age. However, techniques for high resolution *in vivo* retinal imaging like optical coherence tomography (OCT) and adaptive optics (AO) and studies on animal models revealed that ACHM is often associated with progressive cone degeneration [10, 47, 48]. Pathological changes include mild to moderate alterations in cone inner and/or outer segment morphology or in more severe cases substantial loss of foveal cones [47, 48]. In most cases, such degenerative changes can only be observed in aged patients, but were occasionally also seen in younger patients [3, 47-51].

The principle of ACHM gene supplementation therapy relies on the presence of “rescuable” cone photoreceptors, e.g. morphologically intact cones that have not yet entered a progressed

degenerative state, which would not allow them to escape cell death. Therefore, increased amount of natural history data and correlation of clinical with genetic data is needed to ascertain an optimal and accurate definition of the window of opportunity for each candidate gene supplementation patient.

The very encouraging preclinical results have led to the initiation of various translational projects aiming at developing gene supplementation therapies for the more common forms of *CNGA3*- and *CNGB3*-linked ACHM. A phase I/II clinical trial conducted by the RD-CURE consortium encompassing clinicians and researchers from the University Eye Hospital Tübingen and the Ludwig-Maximilians-University Munich was initiated in November 2015 (NCT02610582). The exploratory, dose-escalation study tests safety and efficacy of a single dose subretinal delivery of rAAV8.hCNGA3 to one eye of patients with ACHM caused by *CNGA3* mutations [52]. By the end of 2016 the interventional phase was completed and study results will be reported within 2017. The second ongoing phase I/II clinical trial concerns *CNGB3*-linked ACHM (NCT02599922) and is sponsored by Applied Genetic Technologies Corp (AGTC) in collaboration with the National Eye Institute (NEI). This study also has a non-randomized, open-label and dose-escalation design and tests safety and efficacy of rAAV2tYF-PR1.7-hCNGB3 in patients with ACHM caused by mutations in *CNGB3*. Another phase I/II clinical trial in patients with *CNGA3*-linked ACHM sponsored by AGTC (NCT02935517) is expected to enroll the first patient in 2017.

Outlook

Although there is currently no cure for ACHM, the current research developments provide hope for future treatment of this inherited disorder. Previous activities in the field of retinal gene therapy focused on conditions that were not primarily caused by mutations in

photoreceptor genes. This has led to the pivotal clinical trials in patients with *RPE65*-linked LCA and in Choroideremia (*CHM*) patients [53]. The two ongoing clinical trials in ACHM patients (NCT02610582 and NCT02599922) are now paving the way for the group of inherited eye diseases caused by mutations in photoreceptor genes.

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Conflict of Interest

S.M. and M.B. have submitted patent applications related to AAV gene therapy.

References

1. Aboshiha J, Dubis AM, Carroll J, *et al.* The cone dysfunction syndromes. Br J Ophthalmol 2016; **100**: 115-121. DOI: 10.1136/bjophthalmol-2014-306505
2. Poloschek CM, Kohl S [Achromatopsia]. Ophthalmologe 2010; **107**: 571-580; quiz 581-572. DOI: 10.1007/s00347-010-2178-8
3. Zobor D, Zobor G, Kohl S Achromatopsia: on the doorstep of a possible therapy. Ophthalmic Res 2015; **54**: 103-108. DOI: 10.1159/000435957
4. Biel M, Michalakis S Function and dysfunction of CNG channels: Insights from channelopathies and mouse models. Mol Neurobiol 2007; **35**: 266-277. DOI: 10.1007/s12035-007-0025-y
5. Kohl S, Varsanyi B, Antunes GA, *et al.* CNGB3 mutations account for 50% of all cases with autosomal recessive achromatopsia. Eur J Hum Genet 2005; **13**: 302-308. DOI: 10.1038/sj.ejhg.5201269
6. Shuart NG, Haitin Y, Camp SS, *et al.* Molecular mechanism for 3:1 subunit stoichiometry of rod cyclic nucleotide-gated ion channels. Nat Commun 2011; **2**: 457. DOI: 10.1038/ncomms1466
7. Remmer MH, Rastogi N, Ranka MP, *et al.* Achromatopsia: a review. Current opinion in ophthalmology 2015; **26**: 333-340. DOI: 10.1097/ICU.0000000000000189
8. Chiang WC, Chan P, Wissinger B, *et al.* Achromatopsia mutations target sequential steps of ATF6 activation. Proceedings of the National Academy of Sciences of the United States of America 2017; **114**: 400-405. DOI: 10.1073/pnas.1606387114

9. Biel M, Seeliger M, Pfeifer A, *et al.* Selective loss of cone function in mice lacking the cyclic nucleotide-gated channel CNG3. Proceedings of the National Academy of Sciences of the United States of America 1999; **96**: 7553-7557
10. Michalakis S, Geiger H, Haverkamp S, *et al.* Impaired opsin targeting and cone photoreceptor migration in the retina of mice lacking the cyclic nucleotide-gated channel CNGA3. Investigative ophthalmology & visual science 2005; **46**: 1516-1524. DOI: 10.1167/iovs.04-1503
11. Pang JJ, Deng WT, Dai X, *et al.* AAV-mediated cone rescue in a naturally occurring mouse model of CNGA3-achromatopsia. PloS one 2012; **7**: e35250. DOI: 10.1371/journal.pone.0035250
12. Reicher S, Seroussi E, Gootwine E A mutation in gene CNGA3 is associated with day blindness in sheep. Genomics 2010; **95**: 101-104. DOI: 10.1016/j.ygeno.2009.10.003
13. Shamir MH, Ofri R, Bor A, *et al.* A novel day blindness in sheep: epidemiological, behavioural, electrophysiological and histopathological studies. Veterinary journal 2010; **185**: 130-137. DOI: 10.1016/j.tvjl.2009.05.029
14. Tanaka N, Dutrow EV, Miyadera K, *et al.* Canine CNGA3 Gene Mutations Provide Novel Insights into Human Achromatopsia-Associated Channelopathies and Treatment. PloS one 2015; **10**: e0138943. DOI: 10.1371/journal.pone.0138943
15. Ding XQ, Harry CS, Umino Y, *et al.* Impaired cone function and cone degeneration resulting from CNGB3 deficiency: down-regulation of CNGA3 biosynthesis as a potential mechanism. Human molecular genetics 2009; **18**: 4770-4780. DOI: 10.1093/hmg/ddp440
16. Xu J, Morris L, Fliesler SJ, *et al.* Early-onset, slow progression of cone photoreceptor dysfunction and degeneration in CNG channel subunit CNGB3 deficiency. Investigative ophthalmology & visual science 2011; **52**: 3557-3566. DOI: 10.1167/iovs.10-6358
17. Ding XQ, Thapa A, Ma H, *et al.* The B3 Subunit of the Cone Cyclic Nucleotide-gated Channel Regulates the Light Responses of Cones and Contributes to the Channel Structural Flexibility. J Biol Chem 2016; **291**: 8721-8734. DOI: 10.1074/jbc.M115.696138
18. Sidjanin DJ, Lowe JK, McElwee JL, *et al.* Canine CNGB3 mutations establish cone degeneration as orthologous to the human achromatopsia locus ACHM3. Human molecular genetics 2002; **11**: 1823-1833
19. Yeh CY, Goldstein O, Kukekova AV, *et al.* Genomic deletion of CNGB3 is identical by descent in multiple canine breeds and causes achromatopsia. BMC Genet 2013; **14**: 27. DOI: 10.1186/1471-2156-14-27
20. Dixon CJ Achromatopsia in three sibling Labrador Retrievers in the UK. Vet Ophthalmol 2016; **19**: 68-72. DOI: 10.1111/vop.12265
21. Chang B, Dacey MS, Hawes NL, *et al.* Cone photoreceptor function loss-3, a novel mouse model of achromatopsia due to a mutation in Gnat2. Investigative ophthalmology & visual science 2006; **47**: 5017-5021. DOI: 10.1167/iovs.05-1468
22. Chang B, Grau T, Dangel S, *et al.* A homologous genetic basis of the murine cpfl1 mutant and human achromatopsia linked to mutations in the PDE6C gene. Proceedings of the National Academy of Sciences of the United States of America 2009; **106**: 19581-19586. DOI: 10.1073/pnas.0907720106
23. Brennenstuhl C, Tanimoto N, Burkard M, *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**: 10242-10255. DOI: 10.1074/jbc.M114.611921

24. Kohl S, Zobor D, Chiang WC, *et al.* Mutations in the unfolded protein response regulator ATF6 cause the cone dysfunction disorder achromatopsia. Nat Genet 2015; **47**: 757-765. DOI: 10.1038/ng.3319
25. Kohl S, Hamel C Clinical utility gene card for: Achromatopsia - update 2013. Eur J Hum Genet 2013; **21**. DOI: 10.1038/ejhg.2013.44
26. Daya S, Berns KI Gene therapy using adeno-associated virus vectors. Clinical microbiology reviews 2008; **21**: 583-593. DOI: 10.1128/CMR.00008-08
27. Lipinski DM, Thake M, MacLaren RE Clinical applications of retinal gene therapy. Progress in retinal and eye research 2013; **32**: 22-47. DOI: 10.1016/j.preteyeres.2012.09.001
28. Willett K, Bennett J Immunology of AAV-Mediated Gene Transfer in the Eye. Frontiers in immunology 2013; **4**: 261. DOI: 10.3389/fimmu.2013.00261
29. Boye SE, Boye SL, Lewin AS, *et al.* A comprehensive review of retinal gene therapy. Molecular therapy : the journal of the American Society of Gene Therapy 2013; **21**: 509-519. DOI: 10.1038/mt.2012.280
30. Trapani I, Puppo A, Auricchio A Vector platforms for gene therapy of inherited retinopathies. Progress in retinal and eye research 2014. DOI: 10.1016/j.preteyeres.2014.08.001
31. Urabe M, Ding C, Kotin RM Insect cells as a factory to produce adeno-associated virus type 2 vectors. Human gene therapy 2002; **13**: 1935-1943. DOI: 10.1089/10430340260355347
32. Grimm D, Kern A, Rittner K, *et al.* Novel tools for production and purification of recombinant adenoassociated virus vectors. Human gene therapy 1998; **9**: 2745-2760. DOI: 10.1089/hum.1998.9.18-2745
33. Grieger JC, Choi VW, Samulski RJ Production and characterization of adeno-associated viral vectors. Nature protocols 2006; **1**: 1412-1428. DOI: 10.1038/nprot.2006.207
34. Lisowski L, Tay SS, Alexander IE Adeno-associated virus serotypes for gene therapeutics. Curr Opin Pharmacol 2015; **24**: 59-67. DOI: 10.1016/j.coph.2015.07.006
35. Trapani I, Puppo A, Auricchio A Vector platforms for gene therapy of inherited retinopathies. Progress in retinal and eye research 2014; **43**: 108-128. DOI: 10.1016/j.preteyeres.2014.08.001
36. Dismuke DJ, Tenenbaum L, Samulski RJ Biosafety of recombinant adeno-associated virus vectors. Curr Gene Ther 2013; **13**: 434-452
37. Mingozzi F, High KA Therapeutic in vivo gene transfer for genetic disease using AAV: progress and challenges. Nature reviews Genetics 2011; **12**: 341-355. DOI: 10.1038/nrg2988
38. Kaeppel C, Beattie SG, Fronza R, *et al.* A largely random AAV integration profile after LPLD gene therapy. Nature medicine 2013; **19**: 889-891. DOI: 10.1038/nm.3230
39. Yu H, Mehta A, Wang G, *et al.* Next-generation sequencing of mitochondrial targeted AAV transfer of human ND4 in mice. Molecular vision 2013; **19**: 1482-1491
40. Carvalho LS, Xu J, Pearson RA, *et al.* Long-term and age-dependent restoration of visual function in a mouse model of CNGB3-associated achromatopsia following gene therapy. Human molecular genetics 2011; **20**: 3161-3175. DOI: 10.1093/hmg/ddr218
41. Komaromy AM, Alexander JJ, Rowlan JS, *et al.* Gene therapy rescues cone function in congenital achromatopsia. Human molecular genetics 2010; **19**: 2581-2593. DOI: 10.1093/hmg/ddq136

42. Michalakis S, Muhlfriedel R, Tanimoto N, *et al.* Restoration of cone vision in the CNGA3-/- mouse model of congenital complete lack of cone photoreceptor function. Molecular therapy : the journal of the American Society of Gene Therapy 2010; **18**: 2057-2063. DOI: 10.1038/mt.2010.149
43. Alexander JJ, Umino Y, Everhart D, *et al.* Restoration of cone vision in a mouse model of achromatopsia. Nature medicine 2007; **13**: 685-687. DOI: 10.1038/nm1596
44. Michalakis S, Muehlfriedel RL, Tanimoto N, *et al.* Exploring Different Serotypes And Promoters In rAAV-mediated Gene Replacement Therapy Of Achromatopsia Type 2 (ACHM2). Investigative ophthalmology & visual science 2011; **52**: 490-490
45. Banin E, Gootwine E, Obolensky A, *et al.* Gene Augmentation Therapy Restores Retinal Function and Visual Behavior in a Sheep Model of CNGA3 Achromatopsia. Molecular therapy : the journal of the American Society of Gene Therapy 2015; **23**: 1423-1433. DOI: 10.1038/mt.2015.114
46. Komaromy AM, Rowlan JS, Corr AT, *et al.* Transient Photoreceptor Deconstruction by CNTF Enhances rAAV-Mediated Cone Functional Rescue in Late Stage CNGB3-Achromatopsia. Molecular therapy : the journal of the American Society of Gene Therapy 2013; **21**: 1131-1141. DOI: 10.1038/mt.2013.50
47. Genead MA, Fishman GA, Rha J, *et al.* Photoreceptor structure and function in patients with congenital achromatopsia. Investigative ophthalmology & visual science 2011; **52**: 7298-7308. DOI: 10.1167/iovs.11-7762
48. Thiadens AA, Roosing S, Collin RW, *et al.* Comprehensive analysis of the achromatopsia genes CNGA3 and CNGB3 in progressive cone dystrophy. Ophthalmology 2010; **117**: 825-830 e821. DOI: 10.1016/j.ophtha.2009.09.008
49. Thiadens AA, Slingerland NW, Roosing S, *et al.* Genetic etiology and clinical consequences of complete and incomplete achromatopsia. Ophthalmology 2009; **116**: 1984-1989 e1981. DOI: 10.1016/j.ophtha.2009.03.053
50. Zelinger L, Cideciyan AV, Kohl S, *et al.* Genetics and Disease Expression in the CNGA3 Form of Achromatopsia: Steps on the Path to Gene Therapy. Ophthalmology 2015; **122**: 997-1007. DOI: 10.1016/j.ophtha.2014.11.025
51. Yu XX, Rego RE, Jr., Shechtman D Achromatopsia: case presentation and literature review emphasising the value of spectral domain optical coherence tomography. Clin Exp Optom 2014; **97**: 507-510. DOI: 10.1111/cxo.12175
52. Fischer MD, Wilhelm B, Michalakis S, *et al.* Successful delivery of rAAV8.CNGA3 in a patient with CNGA3 achromatopsia. Investigative ophthalmology & visual science 2016; **57**
53. Scholl HP, Strauss RW, Singh MS, *et al.* Emerging therapies for inherited retinal degeneration. Sci Transl Med 2016; **8**: 368rv366. DOI: 10.1126/scitranslmed.aaf2838
54. Ye GJ, Budzynski E, Sonnentag P, *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. DOI: 10.1089/hum.2015.163
55. Wu J, Rutkowski DT, Dubois M, *et al.* ATF6alpha optimizes long-term endoplasmic reticulum function to protect cells from chronic stress. Dev Cell 2007; **13**: 351-364. DOI: 10.1016/j.devcel.2007.07.005
56. Engin F, Yermalovich A, Nguyen T, *et al.* Restoration of the unfolded protein response in pancreatic beta cells protects mice against type 1 diabetes. Sci Transl Med 2013; **5**: 211ra156. DOI: 10.1126/scitranslmed.3006534
57. Yamamoto K, Sato T, Matsui T, *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**: 365-376. DOI: 10.1016/j.devcel.2007.07.018

Figure Legend

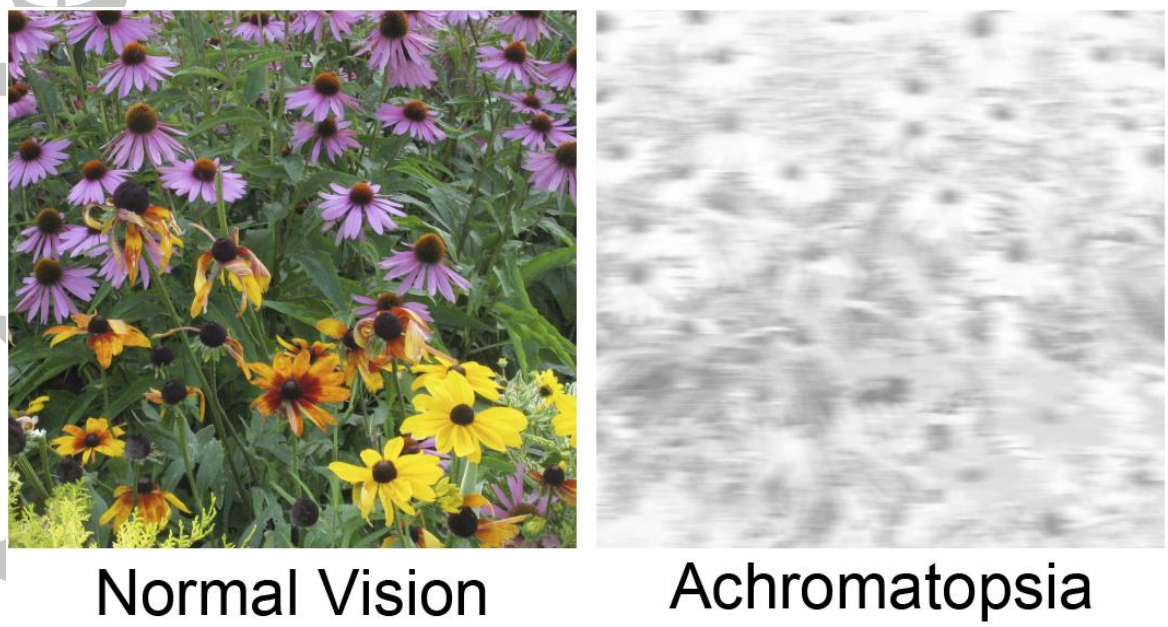

Figure 1. Simulation illustrating daylight vision in Achromatopsia patients. Flower meadow as seen with normal vision at daylight (*left panel*). Patients with Achromatopsia are unable to distinguish colors, have markedly reduced visual acuity and are hypersensitive to ambient light (*right panel*).

Table 1. Current status of gene therapy for Achromatopsia
[1]Online Mendelian Inheritance in Man (omim.org) registry number. [2]Proof-of-concept (POC) studies of gene supplementation therapy in relevant animal models. [3]ClinicalTrails.gov registry number.

Gene	Full Gene Name	OMIM[1]	Animal Models	POC Studies[2]	Clinical Trials[3]
CNGA3	Cyclic nucleotide gated channel alpha 3	600053	Dog [14] Mouse [9] [11] Sheep [12, 13]	[11, 42, 45]	NCT02610582, NCT02935517
CNGB3	Cyclic nucleotide gated channel beta 3	605080	Dog [18-20] Mouse [15, 16]	[40, 41, 46, 54]	NCT02599922
GNAT2	G protein subunit alpha transducin 2	139340	Mouse [21]	[43]	-
PDE6C	Phosphodiesterase 6C	600827	Mouse [22]	-	-
PDE6H	Phosphodiesterase 6H	601190	Mouse [23]	-	-
ATF6	Activating transcription factor 6	605537	Mouse [55-57]	-	-