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# Restoration of cone vision in a mouse model of achromatopsia

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**Loss of cone function in the central retina is a pivotal event in the development of severe vision impairment for many prevalent blinding diseases. Complete achromatopsia is a genetic defect resulting in cone vision loss in 1 in 30,000 individuals. Using adeno-associated virus (AAV) gene therapy, we show that it is possible to target cones and rescue both the cone-mediated electroretinogram response and visual acuity in the *Gnat2*$^{cpfl3}$ mouse model of achromatopsia.**

The human retina has approximately 6 million cone photoreceptors, concentrated predominantly in the central retina, which are responsible for high-resolution and color vision. Complete achromatopsia is a disease involving cones that results in permanent central vision loss, deficient cone-mediated electroretinogram (ERG) signal and color blindness, with affected individuals usually having a visual acuity of 20/200 or less[1–3]. Because these individuals only have functioning rods, they experience extreme light sensitivity and daytime blindness[1–3], owing to the fact that the rods become light saturated. In humans, mutations in cyclic nucleotide–gated channel β-3 (or α-3) and guanine nucleotide α-transducin (*GNAT2*) genes can give rise to this phenotype[1–3]. The *Gnat2*$^{cpfl3}$ homozygous mouse[4] has a single base substitution inducing a missense mutation (D200N) in cone α-transducin, which results in little or no light-adapted (cone-mediated) ERG response and a normal dark-adapted ERG response, similar to observations in the human form of complete achromatopsia[5]. The *GNAT2* mutant form of achromatopsia in humans, and *Gnat2*$^{cpfl3}$ mice, results in a disruption of heterotrimeric G-protein signaling[2,4], which couples light-activated cone visual pigments to the visual transduction cascade.

To develop an AAV vector system that could effectively transduce cones, we first needed a promoter that would efficiently target expression in cones. Previous studies[6,7] have demonstrated that mice transgenic for sequences upstream of the red/green opsin genes, containing a core promoter with a locus control region[6,8,9], can direct the expression of a reporter gene to both classes of cones

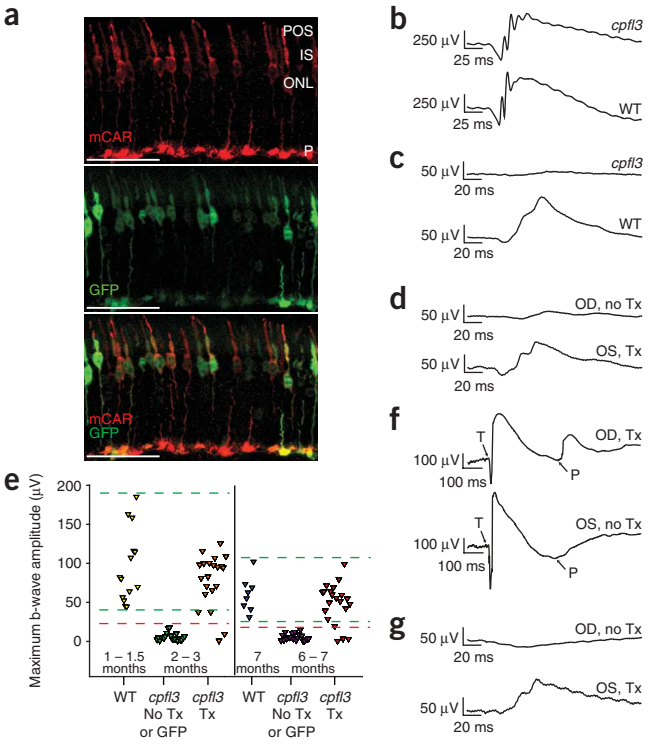


**Figure 1** Cone targeting and rescue of the light-adapted ERG response in *Gnat2*$^{cpfl3}$ mice. (**a**) Confocal fluorescence microscopy depicting antibody staining for mouse cone arrestin (mCAR)[13] colocalizing with GFP expressed from an AAV5-PR2.1-GFP vector in a *Gnat2*$^{cpfl3}$ retinal section. Scale bar, 47 μm. POS, photoreceptor outer segment; IS, photoreceptor inner segment; ONL, outer nuclear layer; P, cone pedicle. (**b**) Representative dark-adapted ERG traces from an untreated 2-month-old *Gnat2*$^{cpfl3}$ mouse (*cpfl3*) and a 1.5-month-old wild-type (WT) mouse. (**c**) As in **b** but for light-adapted ERG response. Dark-adapted ERG traces in response to 0.1 cd·s/m$^2$ flashes, light-adapted ERG traces in response to 10 cd·s/m$^2$ flashes, in the presence of a 100 cd/m$^2$ background light. (**d**) Representative light-adapted ERG traces recorded from the AAV5-PR2.1-*Gnat2*–treated (Tx) and untreated (no Tx) eyes of a 2-month-old *Gnat2*$^{cpfl3}$ mouse; rescue seen only in the treated eye. (**e**) ERG b-wave maximum values recorded from individual *Gnat2*$^{cpfl3}$ eyes, measured at 2 or 3 months of age and then measured again at 6 or 7 months of age. The normal ERG range is the span between the dashed green lines. Values above the dashed red lines were considered to be indicative of a response to therapy. (**f**) Representative paired-flash ERG traces recorded from the treated and untreated eyes of a 3-month-old *Gnat2*$^{cpfl3}$ mouse in response to a rod-saturating test flash (T) and a cone-isolating probe flash (P). Paired-flash ERG traces in response to 0.55 cd·s/m$^2$ flashes. (**g**) Light-adapted ERG traces recorded from the treated and untreated eyes of a 10-month-old *Gnat2*$^{cpfl3}$ mouse treated at 9 months of age. OD, right eye; OS, left eye. The animal studies were approved by the Institutional Animal Care and Use Committees at the University of Florida and SUNY Upstate Medical University.

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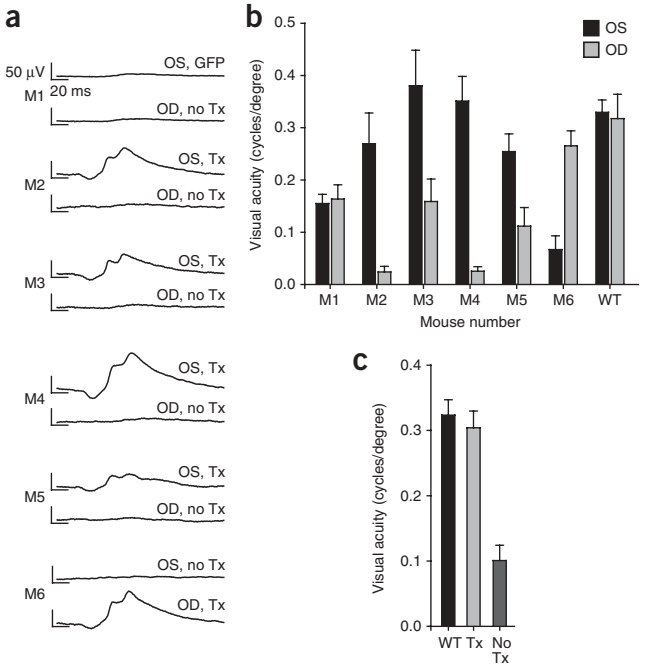


**Figure 2** Rescue of visual acuity in *Gnat2*$^{cpfl3}$ mice. (**a**) Light-adapted ERGs from untreated (no Tx), AAV5-PR2.1-GFP–treated (GFP) and AAV5-PR2.1-*Gnat2*–treated (Tx) *Gnat2*$^{cpfl3}$ eyes, measured at 2 months of age, after vector treatment at 1 month of age (M1–M6, mouse identifiers). OS, left eye; OD, right eye. (**b**) Corresponding visual acuity measurements for the left and right eyes of the six *Gnat2*$^{cpfl3}$ mice (M1–M6) analyzed in **a**, and the average of three wild-type (WT) left and right eyes. Acuities are expressed as the mean (± s.e.m.) for each eye. (**c**) Comparison of the mean (± s.e.m.) visual acuity measurements for all wild-type, treated and untreated (including one GFP control) eyes shown in **b**.

in the mouse retina. On the basis of these considerations, we selected a 2.1-kb human red/green opsin promoter construct (PR2.1) to provide cone specificity for our vector. PR2.1 is composed of bases spanning –4,564 to –3,009 and –496 to 0 of the human red cone opsin gene[6]. When incorporated into an AAV vector and packaged in serotype-5 capsids (AAV5-PR2.1-GFP), this promoter targeted green fluorescent protein (GFP) expression to mouse cones when injected subretinally, as shown by the colocalization of GFP with mouse cone arrestin (**Fig. 1a**). Subsequently, we created an AAV5 vector in which the wild-type mouse cone α-transducin cDNA (*Gnat2*) was under control of the PR2.1 promoter (AAV5-PR2.1-*Gnat2*).

We then injected $4 \times 10^{10}$ vector genome–containing particles of AAV5-PR2.1-*Gnat2* into the subretinal space of *Gnat2*$^{cpfl3}$ mouse eyes, from two litters of 10 and 11 mice, on postnatal days 23 and 29, respectively. The second litter also received $1.5 \times 10^{10}$ vector genomes of AAV5-PR2.1-GFP control vector in the contralateral eye. Because *Gnat2*$^{cpfl3}$ mice have a normal dark-adapted ERG response (**Fig. 1b**) and little or no recordable light-adapted ERG response (**Fig. 1c**), the light-adapted ERG response was our primary measure of efficacy (**Fig. 1d**). Initially, we tested mice between 1 and 2 months after injection. We found that 19 of 21 treated eyes responded to the therapy, and in 17 of these 19, light-adapted ERG amplitudes were at levels seen in the wild-type controls (**Fig. 1e**). Furthermore, between 6 and 7 months of age, 18 of these 21 treated eyes continued to have recordable light-adapted ERG signals, and in 17 of these 18, the signals remained within a normal range (**Fig. 1e**). We defined the normal cone ERG range to be the range of b-wave amplitudes recorded for wild-type mice at similar ages, measured on the same instrument. Whereas rescued eyes did experience an overall decline in b-wave amplitude over time, wild-type mice experienced a similar age-related decline. Thus at least a portion of this loss in treated eyes can be attributed to normal aging effects. In addition, all mice used in this study were albino, and the albino mouse eye is susceptible to light damage[10]. In sum, light-adapted ERG responses were corrected to the normal range in 80% of the vector-treated eyes. As an independent confirmation of cone ERG rescue, we tested a separate group of mice on another ERG-recording device, using the paired-flash method; the results confirmed cone ERG rescue (**Fig. 1f**). To further study the potential of this therapy in older mice, we aged *Gnat2*$^{cpfl3}$ mice to 9 months, then performed injections and analyzed the ERG responses at 10 months. ERG rescue remained feasible in these older mice, and in one case the signal in the treated eye was corrected to a b-wave amplitude of 89 μV (**Fig. 1g**), the level observed in wild-type eyes.

Finally, to determine whether the observed ERG rescue restores a functional measure of vision, we used a behavioral test to determine changes in visual acuity. In another group of *Gnat2*$^{cpfl3}$ mice, each eye received either no injection, subretinal AAV5-PR2.1-GFP or subretinal AAV5-PR2.1-*Gnat2*. At 1 month after injection, we screened these mice by ERG in order to assess rescue (**Fig. 2a**) and selected six mice for behavioral testing. We determined the visual acuity of treated and untreated eyes by measuring optomotor responses to a rotating sine-wave grating[11,12] (OptoMotry, **Supplementary Methods** online). After optomotor testing was complete, the dataset was unblinded, revealing that visual acuity improved only in *Gnat2*$^{cpfl3}$ eyes treated with the AAV5-PR2.1-*Gnat2* vector (**Fig. 2b**). Furthermore, when we compared mean visual acuity measurements (**Fig. 2c**) from all of the wild-type eyes ($\mu = 0.3235$, $n = 6$ eyes) to mean visual acuity measurements from all of the treated eyes ($\mu = 0.3041$, $n = 5$ eyes), the difference was not significant ($P = 0.59$). Thus, visual acuity was restored to levels observed in wild-type mice, demonstrating both the importance of functional cones for mouse visual acuity and the effect of treatment on the visual behavior of the *Gnat2*$^{cpfl3}$ mouse.

To our knowledge, this is the first example of cone-targeted gene therapy correcting cone-mediated ERG function and restoring visual acuity in an animal model of achromatopsia. One caveat is that, because we used human red/green opsin promoter elements, blue cones in humans (~8% of the total cone population) may not be efficiently targeted and a more general cone promoter may be required to completely rescue achromatopsia. In a larger sense, this demonstrates the feasibility of targeting cones in order to treat many of the most prevalent disorders threatening vision in humans. Potential target diseases include cone and cone-rod dystrophies, late-stage retinitis pigmentosa (in which cones are lost after most rods have degenerated), and the exudative forms of age-related macular degeneration and diabetic retinopathy (in which macular cone loss accompanies both macular edema and retinal neovascularization).

*Note: Supplementary information is available on the Nature Medicine website.*

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**AUTHOR CONTRIBUTIONS**

J.J.A. contributed to the design, execution and analysis of all of the experiments, and to manuscript writing. Y.U. contributed to the design and analysis of the behavioral tests. D.E. contributed to the execution and analysis of behavioral and paired-flash ERG tests. B.C. identified and characterized the *Gnat2*$^{cpfl3}$ mutation. S.H.M. and A.M.T. performed the subretinal injections. Q.L. contributed to the AAV-PR2.1 promoter studies. N.L.H. contributed to the characterization of the *Gnat2*$^{cpfl3}$ mouse. J.P. contributed to establishing the mouse colony. R.B.B. contributed to the implementation of behavioral and paired-flash ERG techniques. W.W.H. contributed to the design and analysis of all the experiments, and to manuscript writing.

**COMPETING INTERESTS STATEMENT**

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at www.nature.com/naturemedicine/.

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