

Reply to Townes-Anderson: *RPE65* gene therapy does not alter the natural history of retinal degeneration

We appreciate the interest shown by Townes-Anderson (1) in our article examining the natural history of retinal degeneration in Leber congenital amaurosis caused by retinal pigment epithelium-specific protein 65kDa (*RPE65*) mutations and evaluating the consequences of gene augmentation therapy (2). Townes-Anderson's remarks focused on the final phrase of the last sentence of the *Discussion* of our article. In the full sentence, we suggested that in the future, agents to reduce cell death could be delivered in combination with a more advanced version of the gene augmentation therapy that reaches not only remaining rods and extrafoveal cones but also foveal cone photoreceptors.

Townes-Anderson made the constructive point that consideration should be given in future versions of human *RPE65* gene therapy to a specific rod photoreceptor cell-death pathway (3). We are familiar with the Townes-Anderson hypothesis involving G-protein activation by mislocalized opsin, and we have recently cited this hypothesis as potentially relevant for our work demonstrating successful arrest of retinal degeneration by gene augmentation therapy in the *RPGR* form of X-linked retinitis pigmentosa (4). However, disease mechanisms in the retinoid deficiency caused by *RPE65* mutations and in the ciliopathy caused by *RPGR* mutations are likely to be different. It is important to note that chromophore in *RPE65*-mutant retinas is nearly undetectable and thus the majority of mislocalized opsin molecules are not likely to be bound to chromophore. The protection from bright

light in *Rpe65*-deficient mice—as opposed to exacerbation of degeneration—argues against a primary signaling pathway involving photoactivation of mislocalized isorhodopsin or rhodopsin. Alternatively, it could be argued that mislocalized free opsin is involved. Indeed, there is evidence that constitutive activation of G protein by free opsin contributes to cell death in *Rpe65*-deficiency, but whether signaling originates from free opsin correctly localized to rod outer segments or mislocalized to the inner segments remains unknown.

In patients who received gene augmentation therapy, there is a likely increase in 11-*cis*-retinal chromophore availability to rods and extrafoveal cones (5). According to the Townes-Anderson hypothesis, the increase in chromophore produced by gene therapy together with ambient light should have resulted in activation of mislocalized rhodopsin (1). Such signaling would be predicted to accelerate the rate of cell loss beyond that measured in the natural history of disease. However, the rate of degeneration within the retinal regions that received gene therapy was not faster than the natural history (2), and thus did not support the Townes-Anderson hypothesis.

In summary, we find no specific support in our data for the hypothesis advanced by Townes-Anderson; however, we wholeheartedly agree with the sentiment that we should proceed cautiously to improve the outcomes of retinal gene augmentation therapy including *RPE65* gene therapy.

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1 Townes-Anderson E (2013) Increased levels of gene therapy may not be beneficial in retinal disease. *Proc Natl Acad Sci USA* 110:E1705.

2 Cideciyan AV, et al. (2013) Human retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. *Proc Natl Acad Sci USA* 110(6):E517–E525.

3 Alfinito PD, Townes-Anderson E (2002) Activation of mislocalized opsin kills rod cells: A novel mechanism for rod cell death in retinal disease. *Proc Natl Acad Sci USA* 99(8):5655–5660.

4 Beltran WA, et al. (2012) Gene therapy rescues photoreceptor blindness in dogs and paves the way for treating human X-linked retinitis pigmentosa. *Proc Natl Acad Sci USA* 109(6):2132–2137.

5 Cideciyan AV, et al. (2008) Human gene therapy for *RPE65* isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. *Proc Natl Acad Sci USA* 105(39):15112–15117.

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Conflict of interest statement: W.W.H. and the University of Florida have a financial interest in the use of adeno-associated virus therapies, and own equity in a company (AGTC Inc.) that might, in the future, commercialize some aspects of this work. The University of Pennsylvania, University of Florida, and Cornell University hold a patent on the described gene-therapy technology (United States Patent 20070077228, "Method for Treating or Retarding the Development of Blindness").

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