RPE65 Gene Therapy Restores Melanopsin Function in a Mouse Model of Leber Congenital Amaurosis

Abstract # 72

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Introduction: Many retinal disorders result from disruption of the visual cycle, and while effects of this on rods and cones are well documented, the impact on melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) remains relatively unexplored. There is evidence for compromised melanopsin function in patients with RPE65-specific Leber congenital amaurosis (LCA2) or age-related macular degeneration (AMD), thereby, linking melanopsin function to RPE health. Here, we demonstrate that melanopsin expression and function can be restored in adult mice lacking visual cycle function. **Methods:** *Rd12* mice, which have a mutation in *rpe65*, received a subretinal injection of Adeno Associated Virus (AAV5) encoding *rpe65* in the left eye and an intravitreal injection of the virus in the right eye. Control animals received AAV5 without *rpe65* encoded. After 6 weeks, the pupillary light reflex (PLR) was recorded in dark-adapted animals. Following this, animals were re-dark-adapted and sacrificed by cervical dislocation. Their eyes were enucleated and the intrinsic PLR (iPLR), known to be melanopsin driven, was recorded. Their eyes were fixed, sectioned and stained for RPE65 and melanopsin. Melanopsin labelling intensity in the ON and OFF subdivisions of the inner plexiform layer (IPL) was quantified using pixel value analysis. **Results:** *Rd12* mice, which do not produce RPE65 protein, can not perform the visual cycle and, showed a diminished PLR and iPLR. However, these responses were significantly improved in the left eyes of *rd12* mice which received AAV5 encoding *rpe65* subretinally. This correlated with restoration of RPE65 labelling in the RPE, while RPE65 staining was absent in all other experimental groups. RPE65 labelling in AAV5-*rpe65* subretinal group was restricted to the RPE and not found in other retinal cells. Melanopsin expression is diminished in *rd12* mice, but was partially restored by subretinal delivery of AAV5 encoding *rpe65.* Specifically, melanopsin expression was significantly enhanced in the OFF sublamina of the IPL, directly above regions of RPE65 labelling in the RPE. **Conclusion:** Normal levels of melanopsin expression are supported in adult mice by the visual cycle in RPE cells. Repairing the visual cycle in adult mice lacking RPE65 can reverse the loss of melanopsin expression and improve melanopsin function. These findings have far reaching implications for the treatment of patients with defective RPE, such as LCA2 or AMD.