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Beyond cell death: altered mechanisms and therapy insights in a new mouse model of *GUCA1A* cone-rod dystrophy

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1. Main Text

Cone dystrophies and Cone-Rod dystrophies (CORDs) are severe forms of inherited retinal diseases characterised by progressive degeneration of photoreceptor cells that can lead to legal blindness. To date, no treatment is available to stop the progression of the disease. Photoreceptor viability is strictly dependent on the levels of the second messengers cGMP and Ca^{2+}, the intracellular concentrations of which are finely regulated by guanylate cyclase-activating proteins (GCAPs) and their GC targets. Several mutations in the *GUCA1A* gene coding for GCAP1 have been associated with autosomal dominant CORDs. Among these, the p.(E111V) GCAP1 variant has been shown to dramatically increase intracellular Ca^{2+} and cGMP levels [1,2]. Recently, we confirmed that delivery of the E111V-GCAP1 protein to wild-type mice induces a disease-like electrophysiological phenotype [3], consistent with constitutive cGMP synthesis and increased Ca^{2+} level. In this work, we investigated the role of the E111V-GCAP1variant and its involvement in CORD-related phenotypes in a newly developed knock-in mouse model (E111V) carrying the same human mutation, introduced by genome editing.

2. Methods and results

Methods: Heterozygous and homozygous E111V mouse models and wild-type C57Bl/6 J mice were analysed at different time points to investigate: i) morphological changes in the thickness of both the outer nuclear layer (ONL) and the inner nuclear layer (INL); ii) the differential expression of specific genes; iii) cognitive and behavioural differences. Retina sections were processed for immunofluorescence and immunohistochemistry to assess morphological changes and biodistribution of specific proteins. Retinas from the same animals were homogenised to perform RNA-seq transcriptomic analysis. In addition, each mouse genotype was subjected to a comprehensive behavioural test battery to test cognitive deficits, social behaviour, depth perception and visual-spatial abilities. Electroretinogram (ERG) measurements performed *ex vivo* and local field potential recordings were used to measure the Visually Evoked Potentials (VEP) in the Primary Visual Cortex (V1) and in the Superior colliculus (SC). To assess the therapeutic potential of protein delivery, wild-type GCAP1 was administered *ex vivo* at high concentration in E111V retinas and the effect on photocurrent kinetics measured by ERG.

Results: E111V mice exhibited retinal degeneration with a significantly slower progression than observed in humans, revealing important species-specific differences. Although photoreceptor cell death was relatively modest, ERG recordings in E111V mice showed clear functional alterations, including slowed photoresponse kinetics and increased light sensitivity, consistent with the known effects of the mutation on the GCAP1-GC1 complex.

Behavioral assessments revealed that E111V mice displayed marked deficits in depth perception (Visual Cliff and Pole Cliff test), suggesting impaired visual abilities despite the modest photoresponse alterations observed. This visual impairment was further confirmed by VEP recordings in both V1 and SC, which showed significantly reduced response amplitude and altered latency in E111V mice compared to age-matched controls.

Morphological and immunohistochemical analyses revealed altered retinal distribution patterns of GCAP1 and RD3 proteins in the presence of the CORD mutation, suggesting other roles beside the established ones for these proteins. Transcriptomic analysis indicated that the E111V mutation triggers differential gene expression extending beyond phototransduction pathways to include synaptic and metabolic processes within the retina.

Notably, therapeutic delivery of wild-type GCAP1 demonstrated promising results, suggesting that protein replacement therapy could effectively restore the altered photoresponse kinetics caused by the mutation.

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4. References

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