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**The N-terminal region of centrosomal protein 290 (CEP290) restores vision in a zebrafish model of human blindness.**

Baye, L.M., Patrinostro, X., Swaminathan, S., Bck, J.S., Zhang, Y., Stone, E.M., Sheffield, V.C., & Slusarski, D.C. (2011). The N-terminal region of centrosomal protein 290 (CEP290) restores vision in a zebrafish model of human blindness. *Human Molecular Genetics.* **20** (8): 1467-1477.

## Objectives

 Centrosomal protein 29 (CEP290) has been implicated in the non-syndromic blinding disorder Lebers congenital amaurosis (LCA) as well as several other related cilia based disorders that vary both in severity and tissue involvement. Mutations in CEP290 account for 30% of the cases of LCA and the most common mutation (c.2991 + 1655 A>G) accounts for ~43% of disease causing variants in this gene. The authors investigated the developmental and functional roles of CEP290 in embryonic zebrafish. They tested different regions of CEP290 for localization and ability to restore knockout function. Finally, they tested the ability of the regions to bind NPHP2.

## Experimental Approach & Results

 To observe expression of CEP290 during embryonic and adult development the authors used RT-PCR of all tissues and whole mount *in situ* hybridization to visualize and confirm the presence of CEP290. They found that the gene is maternally inherited and present during all stages of development. The protein was ubiquitously expressed during the 8 somite stage and 5 days post fertilization (dpf) was expressed in the brain, eye, ear, notochord, gut, and retina. Within the retina, expression was observed in the ganglion cell layer, the inner nuclear layer, and the photoreceptor cell layer.

 The authors then modeled the mutation in varying severities using an antisense morpholino injected into 1-4 cell embryos. Knockdown was confirmed with RT-PCR at multiple stages and sequencing of cDNA. This morpholino models the common mutation by disrupting splicing leading to intron inclusion and the generation of a stop codon at amino acid 1024. The characteristic morphology of curling body occurred in a dose dependent fashion. This confirms the known morphology of the mutation as previously demonstrated in both zebrafish and seahorses. The aberrant transcript was present throughout development and some wild-type transcript was also observed.

 Previous research in Bardet-Biedl Syndrome (BBS), another cilia based disorder, has shown abnormal Kupffer vesicle (KV) size and melanosome transport delays in *bbs* gene knockouts. The authors investigated if these characteristics are also present with CEP290 knockdown. They showed a significant percentage of embryos with abnormal KVs and a significant delay in melanosome transport. The abnormal KV size behaved in a dose dependent manner while the melanosome transport did not.

 LCA patients present with normal retinal lamination and visual impairment and so the authors investigated whether this was reflected in the model. Using the 8ng dose to generate differing body morphologies (curly and straight) and found that regardless of phenotype at 3dpf the embryos had fully laminated retinas and normal outer photoreceptor cells. At 5 dpf they observed that the outer cells were beginning to show some signs of cellular disorganization. In order to asses vision, the authors utilized a natural escape response upon change in light intensity. They used only straight body morphology and *crx* mutants as a positive impaired control. They found significantly reduced visual acuity in the morphants similar to the *crx* mutants.

 The authors’ goal was to test human constructs of the N-terminal region (1-1059AA) and the C-terminal region (1765-2479AA) in their ability to localize to centrioles and restore function. The N construct extends over the modeled mutation and the C construct extends over two other mutations identified in other cilia based disorders. In order to test for localization they used mRNA encoding myc-tagged fusion constructs injected into embryo. Immuno-staining was used to confirm expression of the constructs and centrin with GFP was coinjected to visualize localization. They observed both constructs localizing to the paracentriolar region and in cytoplasm. Upon coinjection of the morpholino and the constructs and a visual acuity test they found that only the N construct was sufficient to restore visual functions as shown by the N treatment group having statistically similar visual acuity to controls. The C construct alone rescued melanosome transport delays, but neither construct was able to rescues KV defects.

 It has been shown in previous research that the NPHP proteins (including CEP290) can form complexes and that mutations in other NPHP proteins have been implicated in syndromic blinding disorders. The authors tested whether either of the constructs would bind with NPHP2 using transient transfection and co-immunoprecipitation assays. They found that only the N-terminal construct interacts with NPHP2.

## Conclusion

 The authors determined that CEP290 is present throughout development and is present in multiple tissues. Their model demonstrated body curve defects, decreased KV size, delayed melanosome transport, and vision impairment with normal retinal lamination. They showed that an N-terminal construct was sufficient to restore vision and that it interacts with other proteins known to play a role in proper vision, specifically NPHP2. The dose dependence of the defects and the presence of wild type transcript further support the hypothesis that LCA is a hypomorphic mutation.

## Future Research & Critique

Overall this paper is very strong in that it demonstrates known phenotypes for this mutation and uses multiple methods to confirm their mutation. They investigate a novel question that has implications in future treatment options for this blinding disorder. The authors acknowledge that their research negates previous work with the *rd16* mouse and rdAc cat in that the C-terminal region is implicated in loss of vision. The authors postulate the C region mutations in these models cause instability or misfolding in the protein affecting the N region as well. Also, in the *rd16* mouse the abnormal CEP290 localizes aberrantly to the photoreceptor inner segment. However they didn’t explain how this mutation models BBS or why this was important to this research. What mutations result in BBS and how is LCA or CEP290 related to BBS other than being cilia based disorders?

If I were to continue this research I would look into identifying NPHP complexes and proteins with which they interact during proper vision. This could elucidate the molecular pathways that enable proper vision. This would have to be done in a tissue specific manner because these proteins appear to have tissue specific functions. Detailed knowledge of the pathways involved in vision may increase understanding of the mechanisms of these diseases. I would also like to test this mutation and rescue in a different model that is genetically more similar to humans such as a murine model. If successful, it may offer a more refined treatment approach to LCA.

The authors suggest identifying other proteins that interact specifically with CEP290 to help explain rescue by N-terminus thus identifying the specific mechanism of action of the N-terminal region of CEP290.