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"The Effect of R81I and V240F Mutations on Protein Expression and Enzymatic Activity of RPE65"

Retinal pigment epithelium-specific 65 kDa protein (RPE65) is a retinoid isomerase, catalyzing conversion of all-trans-retinyl esters to 11-cis-retinol, a key step in regenerating light-sensitive visual pigments in rod and cone photoreceptor neurons of the retina. Mutations in the RPE65 gene are linked to retinal degenerative diseases, such as Leber congenital amaurosis (LCA), early-onset retinal dystrophy (EORD) and retinitis pigmentosa (RP). Recent research identified R81I and V240F mutations of RPE65 as potential pathogenic mutations associated with LCA and EORD. However, the pathogenicity and mechanisms of these two mutations remains unknown. This study focused on the impact of these two mutations on RPE65 protein expression and enzymatic activity. We hypothesized that these mutations might disrupt the enzyme's active site or structure, leading to a loss of function.

To test our hypothesis, we introduced individually R81I and V240F mutations into wild-type RPE65 cDNA through site-directed mutagenesis. These cDNAs were cloned in the pRK5 plasmid, a mammalian expression vector. HEK293C cells were transfected with either wild type, pRK5 (negative control), or mutant RPE65 DNA, and protein expression levels were assessed using polyacrylamide gel electrophoresis and Western blot analysis. We observed that immunoblot intensities of R81I and V240F mutant bands, visualized and normalized to the internal marker protein (actin) using Image Quant LAS 4000, were comparable to that of the wild type RPE65. These findings indicate that the two mutations have no significant effect on the RPE65 protein expression and its stability in the cells.

Given that the mutations do not impair protein synthesis or stability, the pathogenicity likely arises from their impact on enzymatic function. Preliminary data of enzymatic assays suggests that the R81I mutation almost completely eliminated the retinoid isomerase activity of RPE65, while the V240F mutation dramatically reduced it. Next steps involve performing assays to quantify this impairment and identify ways to rescue the isomerase function of these mutants using chemical chaperones or other approaches.

In conclusion, R81I and V240F mutations do not alter RPE65 protein expression but likely affect its enzymatic activity. Further research is needed to understand the pathogenic mechanisms and explore potential therapies.