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

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Introduction

RPE65 (Retinal pigment epithelium-specific 65 kDa protein) is a crucial enzyme in the visual cycle, responsible for converting all-trans-retinyl esters to 11-cis-retinol. This conversion is essential for regenerating light-sensitive visual pigments in the retina, specifically in rod and cone photoreceptor neurons. Mutations in the RPE65 gene are associated with several retinal degenerative diseases, including Leber congenital amaurosis (LCA) and early-onset retinal dystrophy (EORD), leading to vision loss.

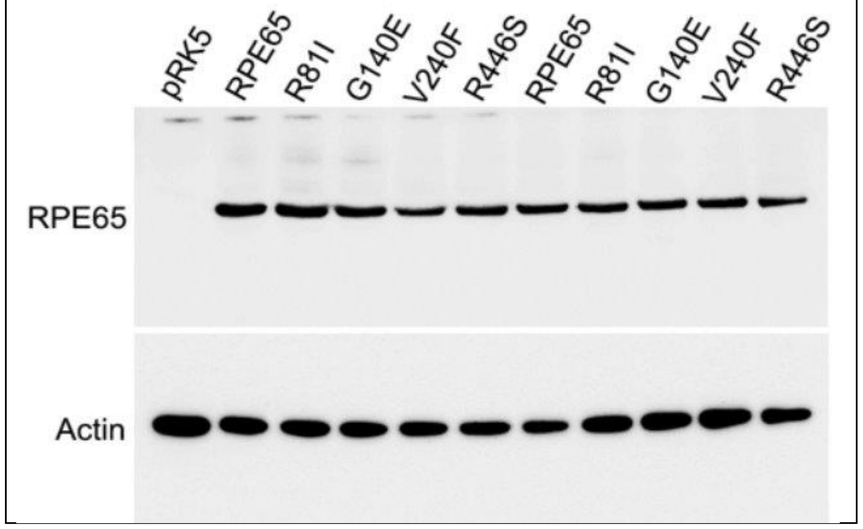
Recent research has identified R81I and V240F mutations as likely pathogenic. Our hypothesis is that these mutations disrupt the enzymatic function of RPE65 without significantly altering protein expression levels. To test this, we employed site-directed mutagenesis to introduce the mutations into RPE65 cDNA and assessed their effects on protein expression and enzymatic activity using transfected HEK293C cells.

Methods

- Proteins of 293T-LRAT cells transfected with wild type (WT) and mutant plasmids were separated in a 12% gel by SDS-PAGE and then transferred onto a membrane using a Semi-Dry transfer for Western blot analysis with primary and secondary antibodies.
- Immunoblots of WT and mutant RPE65 were visualized and normalized with an internal marker Actin using ImageQuant LAS 4000.
- The retinoid isomerase activity of WT and mutant RPE65 was determined by monitoring the synthesis of 11-*cis*-retiol from all-*trans*-retinol substrate in the 293T-LRAT cells expressing WT or mutant RPE65 protein.

Immunoblot Intensities

***Figure 1.1**: Protein expression in 293T-LRAT cells transfected with indicated plasmids, normalized to actin*



Retinoid Isomerase Assays

***Figure 2.1:** HPLC showing reduced 11-cis-retinol formation in 293T-LRAT cells expressing the V240F mutant RPE65 compared to wild-type*

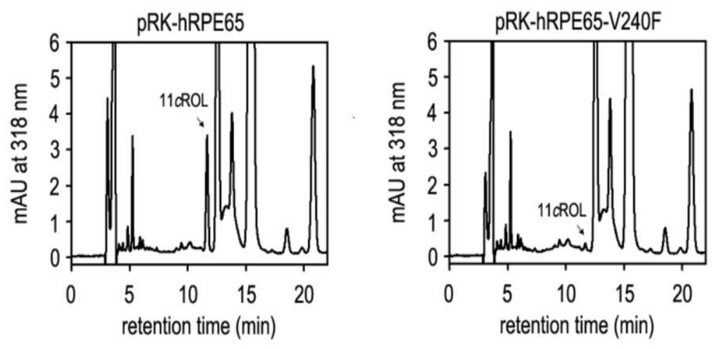
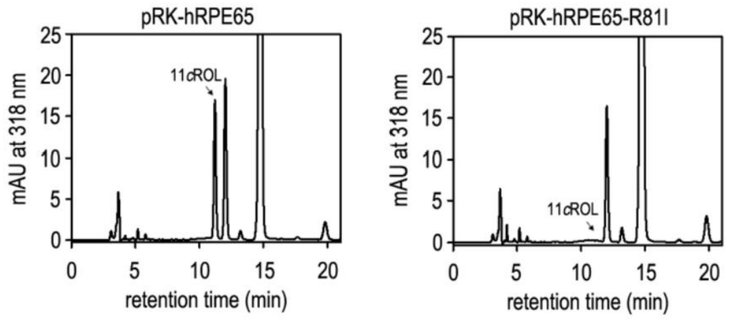
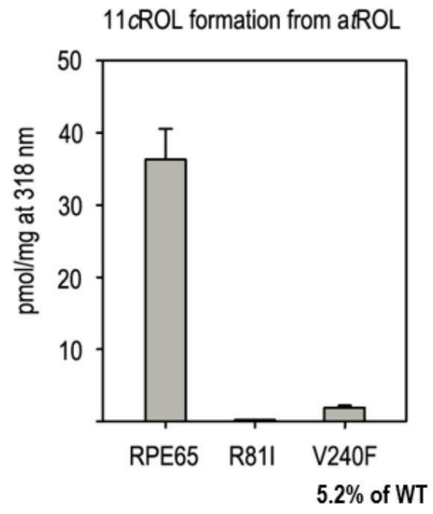


***Figure 2.2:** HPLC showing no detectable 11-cis-retinol in 293T-LRAT cells expressing R81I-mutant RPE65, compared to wild-type*



Quantitative Analysis of Assays

***Figure 3.1:** Quantitative analysis of 11-cis-retinol levels in 293T-LRAT cells*



Conclusion

While the R81I and V240F mutations do not affect RPE65 protein expression levels, they significantly impair the enzymatic activity of RPE65.
The lack of functional enzyme activity suggests these mutations contribute to the pathogenicity observed in associated retinal diseases.

Future research will focus on potential therapeutic strategies to rescue the enzymatic function of these mutant proteins, such as using chemical chaperones or alternative approaches. Understanding these mechanisms could lead to novel treatments for retinal degenerative diseases linked to RPE65 mutations.