Disease mechanism of RPE65 mutations observed in Costa Rican children with retinal dystrophies

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Introduction

- RPE65 is an important retinoid isomerase in the visual cycle.
- RPE65 regenerates 11-*cis*-retinal, the light sensor of the opsin visual pigments essential for initiating phototransduction in the retinal rod and cone photoreceptor neurons in response to light stimuli.
- Biallelic RPE65 mutations have been found in Costa Rican children with Leber congenital amaurosis (LCA) or early-onset retinal dystrophy (EORD).

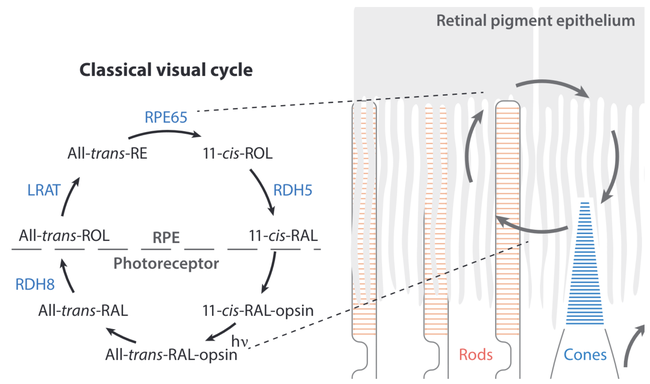


Figure 1. The visual cycle comprises enzymes in the retinal pigment epithelium (RPE). RPE65 coverts all-trans-RE to 11-cis-ROL in the visual cycle. *Image from:* Kiser, P. D., & Palczewski, K. (2016). Retinoids and retinal diseases. *Annual Review of Vision Science*, *2*(1), 197–234. https://doi.org/10.1146/annurev-vision-111815-114407

- **Purpose:** To determine the effect of G140E and R446S mutations found in Costa Rican children on the stability and enzymatic function of RPE65 isomerase.
- **Method:** Using PCR method combined with a site-directed mutagenesis kit, we introduced G140E and R446S mutations individually into the wild-type RPE65 gene that has been cloned in the pRK5 plasmid, a mammalian expression vector. The mutations and coding region of RPE65 were confirmed by DNA sequencing. Stability of wild-type and mutant RPE65s was assessed by immunoblot analysis in HEK293T-LC cells stably expressing LRAT, an enzyme that makes the substrate of RPE65. Retinoid isomerase assay was used to determine the enzymatic activity of wild-type, G140E mutant RPE65, and R446S mutant RPE65 by measuring the synthesis of 11-*cis*-retinol from all-*trans*-retinol.

G140E and R446S mutants

Figure 2. DNA sequence analysis of G140E-RPE65 mutant cloned in pRK5 vector. At position 140, Gly (G) from GGG changed to Glu (E) GAG.

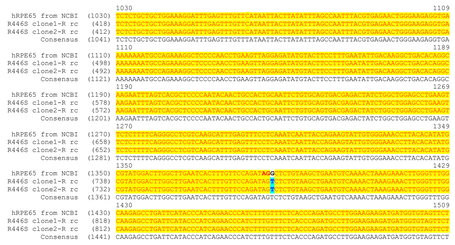


Figure 3. DNA sequence analysis of R446S-RPE65 mutant cloned in pRK5 vector. At position 446, Arg (R) from AGG changed to Ser (S) AGT.

RPE65 expression

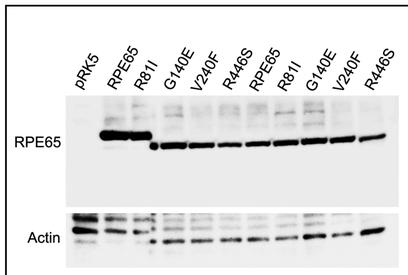


Figure 4. Western blot analysis of 293T-L cells transfected with the indicated plasmid

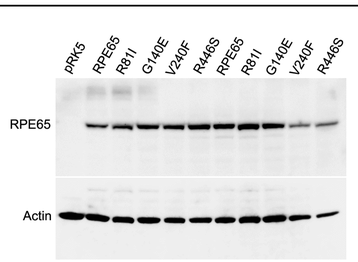


Figure 5. Western blot analysis of 293T-L cells transfected with the indicated plasmid (done by all members)

Quantitative analysis

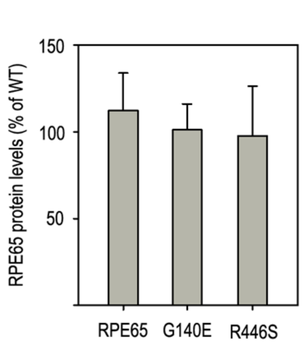


Figure 6. Quantitative analysis of RPE65 protein levels in 293T-L cells expressing wild-type human RPE65 or the indicated RPE65 mutant. Expression levels of the G140E- and R446S-RPE65 mutants were similar to that of wild-type RPE65 in the HEK293T-LC cells.

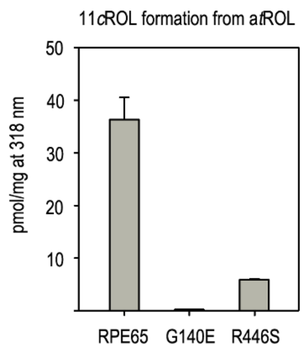
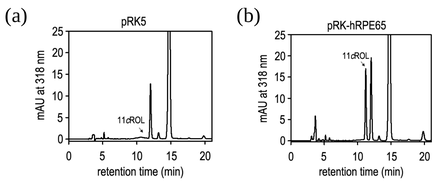


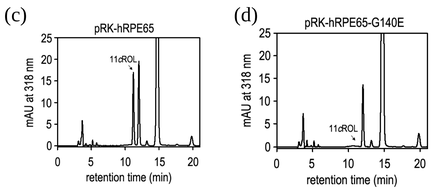
Figure 7. Quantitative analysis of 11-*cis*-retinol in 293T-L cells expressing wild-type human RPE65 or the indicated RPE65 mutant. RPE65 with the G140E mutation almost completely lost isomerase function, while isomerase activity of R446S-RPE65 was less than 20% of wild-type RPE65's activity.

Retinoid isomerase activity



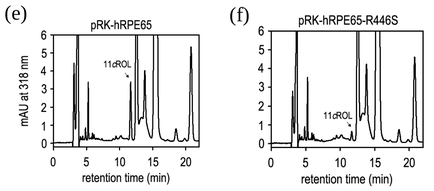
Negative and positive controls of the retinoid isomerase assay.

Figure 8. HPLC chromatograms showing 11-*cis*-retinol formation from all-*trans*-retinol incubated with the 293T-L cells that have been transfected with pRK5 (a) or pRK-hRPE65 (human RPE65) plasmid DNA (b). Note that cells transfected with pRK5 mock vector did not produce 11-*cis*-retinol in the same conditions (a).



G140E mutation completely abolished enzymatic function of RPE65 isomerase.

Figure 9. HPLC chromatograms showing lack of detectable 11-*cis*-retinol in the 293T-L cells expressing human RPE65 with G140E mutation (d), as compared to the cells expressing wild-type human RPE65 (c).



R446S mutation significantly reduced isomerase activity of RPE65.

Figure 10. The amounts of 11-*cis*-retinol synthesized in the 293T-L cells expressing human RPE65 with R446S mutation (f) were significantly smaller than those in the cells expressing wild-type hRPE65 (e).

Conclusion

- G140E and R446S mutations did not significantly reduce the stability of RPE65 isomerase.
- However, both G140E and R446S mutations dramatically reduced the enzymatic function of RPE65 isomerase, indicating that both are disease-causing mutations.
- The G140E mutation may have a stronger pathogenic effect compared to the R446S mutation.
- Further studies are needed to confirm our findings and to elucidate the molecular basis for the phenotypic difference between the two mutations.

References:

Glen WB.Jr., Peterseim MW, Badilla R, Znoyko I, Bourg A, Wilson R, Hardiman G, Wolff D & Martinez J (2019) A high prevalence of biallelic RPE65 mutations in Costa Rican children with Leber congenital amaurosis and early-onset retinal dystrophy, *Ophthalmic Genetics*, 40:110-117, DOI: 10.1080/13816810.2019.1582069

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