

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

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.

Retinoid isomerase activity



Negative and positive controls of the retinoid isomerase assay.

Figure 8. HPLC chromatograms showing 11-cis-retinol formation from alltrans-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

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

020		
USU 	(1030)	hPDF65 from NCBI
	(1030)	R446S clopel-R rc
	(412)	R446S clone2-B rc
CTCTGCTGCTGCAAAGGATTTGAGTTTGTTTATAATTACTTATATTTAGCCAATTTACGTGAGAACTGGGAAGAGGTC	(1041)	Consensus
110 118	(,	
AAAAAATGCCAGAAAGGCTCCCCAACCTGAAGTTAGGAGATATGTACTTCCTTTGAATATTGACAAGGCTGACACAGG	(1110)	hRPE65 from NCBI
AAAAAATGCCAGAAAGGCTCCCCAACCTGAAGTTAGGAGATATGTACTTCCTTTGAATATTGACAAGGCTGACACAGG	(498)	R446S clonel-R rc
AAAAAATGCCAGAAAGGCTCCCCAACCTGAAGTTAGGAGATATGTACTTCCTTTGAATATTGACAAGGCTGACACAGG	(492)	R446S clone2-R rc
AAAAAATGCCAGAAAGGCTCCCCAACCTGAAGTTAGGAGATATGTACTTCCTTTGAATATTGACAAGGCTGACACAGG	(1121)	Consensus
190 126		
<mark>AGAATTTAGTCACGCTCCCCAATACAACTGCCACTGCAATTCTGTGCAGTGACGAGACTATCTGGCTGG</mark>	(1190)	hRPE65 from NCBI
AGAATTTAGTCACGCTCCCCAATACAACTGCCACTGCAATTCTGTGCAGTGACGAGACTATCTGGCTGG	(578)	R446S clonel-R rc
AGAATTTAGTCACGCTCCCCAATACAACTGCCACTGCAATTCTGTGCAGTGACGAGACTATCTGGCTGG	(572)	R446S clone2-R rc
AGAATTTAGTCACGCTCCCCAATACAACTGCCACTGCAATTCTGTGCAGTGACGAGACTATCTGGCTGG	(1201)	Consensus
270	(1070)	
	(1270)	nRPE65 from NCBI
	(652)	R4405 Clonel-R IC
	(1281)	Consensus
350	(1201)	consensus
GTATGGACTTGGCTTGAATCACTTTGTTCCAGAT AG CTCTGTAAGCTGAATGTCAAAACTAAAGAAACTTGGGTTTG	(1350)	hRPE65 from NCBI
GTATGGACTTGGCTTGAATCACTTTGTTCCAGATAG <mark>T</mark> CTCTGTAAGCTGAATGTCAAAACTAAAGAAACTTGGGTTTG	(738)	R446S clonel-R rc
GTATGGACTTGGCTTGAATCACTTTGTTCCAGATAG <mark>T</mark> CTCTGTAAGCTGAATGTCAAAACTAAAGAAACTTGGGTTTG	(732)	R446S clone2-R rc
GTATGGACTTGGCTTGAATCACTTTGTTCCAGATAGTCTCTGTAAGCTGAATGTCAAAACTAAAGAAACTTGGGTTTG	(1361)	Consensus
430 150		
AAGAGCCTGATTCATACCCATCAGAACCCATCTTTGTTTCTCACCCAGATGCCTTGGAAGAAGATGATGGTGTAGTTC	(1430)	hRPE65 from NCBI
AAGAGCCTGATTCATACCCATCAGAACCCATCTTTGTTTCTCACCCAGATGCCTTGGAAGAAGATGATGGTGTAGTTC	(818)	R446S clone1-R rc
AAGAGCCTGATTCATACCCATCAGAACCCATCTTTGTTTCTCACCCAGATGCCTTGGAAGAAGATGATGGTGTAGTTC	(812)	R446S clone2-R rc
	(1 / / 1)	Conconcus

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



in the same conditions (a).



G140E mutation completely abolished enzymatic function of RPE65

Figure 9. HPLC chromatograms showing lack of detectable 11-cisretinol 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).

- **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

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

150

100

50

of WT)

ls (%

prote

RPE65

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 wildtype 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 wildtype RPE65's activity.

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

measuring the synthesis of 11-cis-retinol from

all-*trans*-retinol.

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