Study of the disease mechanism of RPE65 mutations observed in Costa Rican children with retinal dystrophies

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Background: RPE65 is an important retinoid isomerase in the visual cycle that 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. Mutations in RPE65 have been known to cause blinding diseases such as Leber congenital amaurosis (LCA), early-onset retinal dystrophy (EORD) or retinitis pigmentosa. Biallelic RPE65 mutations have been found in Costa Rican children with LCA or EORD. However, pathogenicity and the molecular pathogenic mechanism of these mutations remain unknown.
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 RPE65 by measuring the synthesis of 11-*cis*-retinol from all-*trans*-retinol, incubated with cells transfected with pRK5 mock vector and WT or mutant RPE65 constructs.
Results: Immunoblot analysis showed that expression levels of the G140E- and R446S-RPE65 mutants were similar to that of wild-type RPE65 in the HEK293T-LC cells. However, 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.
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

- Li S, Samardzija M, Yang Z, Grimm C, Jin M (2016) Pharmacological Amelioration of Cone Survival and Vision in a Mouse Model for Leber Congenital Amaurosis, *J Neurosci*. 36:5808-19. doi: 10.1523/JNEUROSCI.3857-15.2016.