Original Article

A Gene Scan Study of *RPE65* in Chinese Patients with Leber Congenital Amaurosis

Jing Liu, Juan Bu

Eye Department, Peking University Third Hospital, Beijing Key Laboratory of Restoration of Damaged Ocular Nerve, Beijing 100191, China

Abstract

Background: Leber congenital amaurosis (LCA) is a visual disease which is caused by *RPE65* mutations and results in retinal degeneration and severe vision loss in early infancy. According to previous researches, mutations of the *RPE65* gene account for 16% of all cases of LCA. This study aimed to identify *RPE65* gene mutations in Chinese patients with LCA.

Methods: We recruited 52 sporadic patients from Peking University Third Hospital in 2016 and applied Sanger sequencing to identify variants among exons responsible for the disease. The genomic DNAs from blood leukocytes of these patients were isolated, and the entire coding region of the *RPE65* gene was amplified by polymerase chain reaction. We then determined the sequence of *RPE65* using ABI 3100 Genetic Analyzer.

Results: Our study identified that only 1 out of the 52 patients with LCA carried the previously unreported homozygosis missense mutation c1174A>C (T392P) of the *RPE65* gene. However, the mutation was associated with the disease phenotype and not detected in 100 normal controls.

Conclusions: Though we identified a novel missense mutation in the *RPE65* gene that causes LCA, our result indicates that *RPE65* mutations may not play a major role in the LCA patients in China since only 1 out of the 52 patients carried mutation in the *RPE65* gene.

Key words: Leber Congenital Amaurosis; Mutation; *RPE65*

Introduction

Leber congenital amaurosis (LCA) is the most common genetic cause of congenital visual impairment in children and infants, and is characterized by a severe dystrophy of the retina. LCA affects around 1 in 80,000 of the population. Visual function of LCA patients is usually poor and often accompanied by nystagmus, sluggish or near-absent pupillary responses, photophobia, high hyperopia, and keratoconus. There are 17 genes, including the *RPE65* gene, known to cause LCA, and mutations in these genes account for at least half of the LCA cases. Mutation of the *RPE65* gene may be associated with LCA type 2 (LCA2), which causes night blindness. *RPE65* contains 14 coding exons and encodes a protein of 65,000 expressing specifically and abundantly in the retinal pigment epithelium (RPE), which is involved in the production of 11-cis retinal and visual pigment regeneration.[1,2] Clinical trials using *RPE65* as the only targeting molecule for LCA gene therapy are progressing rapidly recently. According to a research by Morimura *et al*.,[3] mutations of the *RPE65* gene account for 16% of the cases of LCA. In the case of LCA2, though some patients may experience transient improvement in vision, they eventually progress to a complete vision loss.[4,5]

While LCA has been identified as a major cause of congenital visual impairment, the prevalence of the disease varies across different geographical origins.[6,7] The purpose of this study was to analyze *RPE65* mutation in Chinese patients with LCA, which may provide useful information for gene therapy of this disease in China.

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.218007

Address for correspondence: Dr. Juan Bu, Eye Department, Peking University Third Hospital, Beijing Key Laboratory of Restoration of Damaged Ocular Nerve, Beijing 100191, China
E-Mail: bujuan110@163.com

Received: 08-08-2017 **Edited by:** Yuan-Yuan Ji
How to cite this article: Liu J, Bu J. A Gene Scan Study of *RPE65* in Chinese Patients with Leber Congenital Amaurosis. Chin Med J 2017;130:2709-12.

Methods

The study was conducted in accordance with the *Declaration of Helsinki* and approved by the local ethics committee of Peking University Third Hospital (No. 2012093). Informed written consent was obtained from all patients prior to their enrollment in this study.

Clinical data and 4-ml blood samples were collected from patients with LCA. The patients underwent complete physical and ophthalmic examinations. To identify causative mutations, genomic DNA was extracted from peripheral blood cells according to standard protocol (Roche Diagnostics Corporation, Indianapolis, USA). Then, all the exons and exon-intron boundaries of *RPE65* were amplified using the standard polymerase chain reaction (PCR) buffer system with primers [Table 1]. PCR reactions were each performed in a 10 μl volume containing 1.5 mmol/L $MgCl_2$, 0.4 mmol/L of each primer, 200 μmol/L dNTPs, 1 U Taq DNA polymerase (Takara, Japan), and 10–20 ng template DNA. Amplification was performed with an initial denaturation for 3 min at 95°C, followed by 30 cycles of denaturation at 95°C for 1 min; we then annealed at 55°C for 1 min with extension at 72°C for 1 min, and a final extension at 72°C for 3 min.

PCR products were purified using a PCR product purification kit (Qiagen, CA). Purified PCR products were sequenced using the BigDye Terminator Cycle Sequencing v3.1 kit (Applied Biosystems, CA, USA). Then, 10 ng of template DNA was added in each reaction followed by a temperature program which included 25 cycles of denaturation at 97°C for 30 s, annealing at 50°C for 15 s, and an extension at 60°C for 4 min. All samples were analyzed in an ABI Prism 310 Genetic Analyzer (Applied Biosystems, CA, USA). The *RPE65* cDNA reference sequence with GenBank accession No. NC_000001.10 was used (National Center for Biotechnical Information, Bethesda, Md; available at: http://www.ncbi.nlm.nih.gov).

We predicted the protein structure via the threading approach. Both protein sequences were searched against PDB database to select the most similar templates along with sequence-structured alignment. Given the candidate templates and target-template alignments, a modeler was used to build candidate models for each corresponding template.

Results

Totally 52 sporadic LCA patients were recruited. All patients have early severe visual deficits in childhood with their visual acuity <20/400. Sequencing of the 14 coding exons of *RPE65* identified a mutation in exon 11 [c.1174 A > C, Figure 1a] in one patient, which resulted in substitution of threonine by proline (T392P). The mutation was not found in other patients and 100 ethnic unrelated and unaffected normal controls [Figure 1b].

The mutation led to a significant change in the RPE65 protein's structure. For each model, we observed difficulties in obtaining the most stable tertiary structure of the side chain structures of each amino acid [Figure 2].

The *RPE65* mutation patient was a 23-year-old male without a family history of LCA. The disease appeared when he was 17 years and his vision decreased to 0.01 gradually. Pendular nystagmus and deep-set eyes were found in this patient, who was extremely sensitive to light. The results of fundus examination displayed a salt-and-pepper appearance with minimal attenuated retinal vessels, and many whitish punctuate lesions in the midperipheral retina [Figure 3]. Extinguished electroretinogram was observed [Figure 4].

Table 1: Primers used to amplify the exons of *RPE65*

Primer name	Sequence	Melting temperature (°C)	Product size (bp)
RPE65_E1_F	aagcaacttctgttcccct	60.11	308
RPE65_E1_R	tttccccaccaaaattcaag	59.77	
RPE65_E2_F	ggagtgaacaggctttgagc	60.00	324
RPE65_E2_R	aaaccacctgatccctctcc	60.31	
RPE65_E3_F	cactgccagctctatgagga	59.14	410
RPE65_E3_R	actggcccaggtacattgtg	60.83	
RPE65_E4/5_F	tttatgattgtgacttgatgagga	58.63	367
RPE65_E4/5_R	catttggagcttggaatggt	59.93	
RPE65_E6_F	aggatgagagttcaaggggt	57.62	402
RPE65_E6_R	atagggtagggatgagggca	60.67	
RPE65_E7/8/9_F	tcaaaatgtgtttctttgcct	57.41	900
RPE65_E7/8/9_R	tttgactctcacataactcttgctg	60.00	
RPE65_E10_F	agcagtttctgggttgtgga	60.69	379
RPE65_E10_R	gcctatttttaaagctccttctagc	59.55	
RPE65_E11/12/13_F	tccctgcatgttgacctaaa	59.12	826
RPE65_E11/12/13_R	ggatcgtttttgagtattacgga	59.41	
RPE65_E14_F	tcaggtcatatggttttctatatttg	57.75	499
RPE65_E14_R	ggcctgtctcacagaggaag	59.99	

RPE: Retinal pigment epithelium.

Discussion

LCA accounts for at least 5% of all retinal dystrophies and is one of the main causes of blindness in children.[8,9] Missense mutations in *RPE65* were identified in a patient with LCA2 using the candidate gene scanning approach.[10] Since the initial report, a wide range of *RPE65* mutations associated with LCA had been identified.[5,11,12] The RPE65 protein has an essential role in maintaining retinal function and photoreceptor viability, and mutations in this protein affect the essential pathways involved in the processing and metabolism of Vitamin A and retinoid cycling between the RPE and photoreceptors.[13]

Young patients with *RPE65* mutations display a foveal cone loss along with shortened inner and outer segments of the remaining cones. Maeda *et al*.[14] suggested that chronic lack of chromophore might lead to a progressive loss of cones in mice and humans, and that therapy for LCA patients could be geared toward early adequate delivery of chromophore to cone photoreceptors. *RPE65* was the

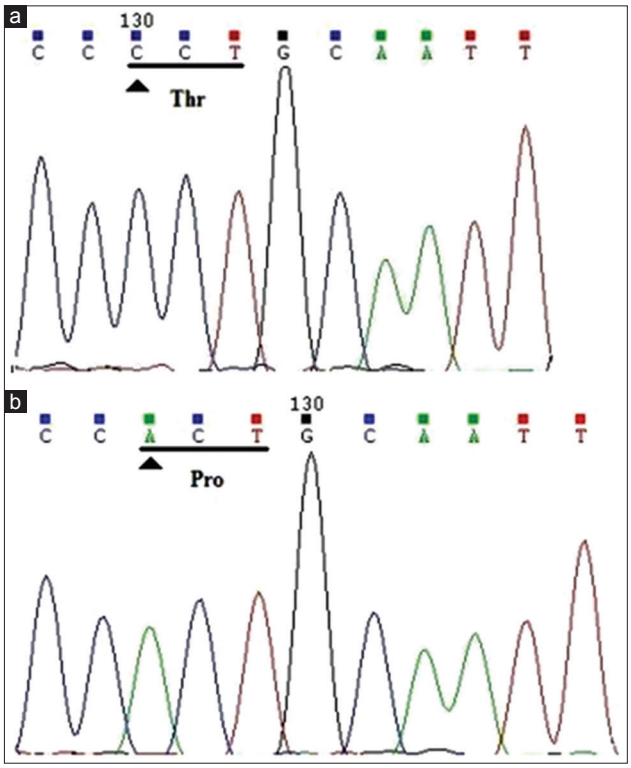

Figure 1: Reversed sanger result of the *RPE65* missense mutation in extron 11. (a) A homozygosis change c1174A>C in *RPE65* (indicated by the black arrowhead) was identified in one of the LCA patients. (b) The corresponding normal sequence in other LCA patients and in the normal controls. LCA: Leber congenital amaurosis.

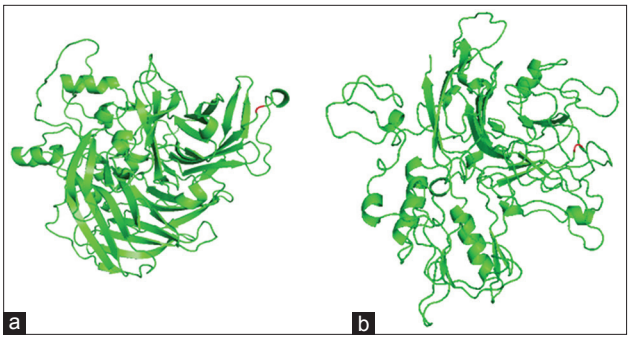

Figure 2: Comparison of RPE65 structure model. (a) Before mutation, (b) after mutation.

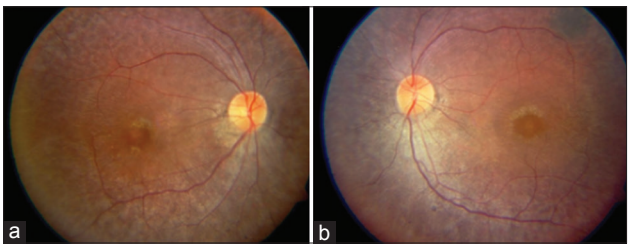

Figure 3: Color fundus photographs of the eyes of the patient with the uncommon mutation of *RPE65*. (a) The right eye, (b) the left eye. The fundus photographs displayed a salt-and-pepper appearance with minimal attenuated retinal vessels, and many whitish punctuate lesions in the midperipheral retina.

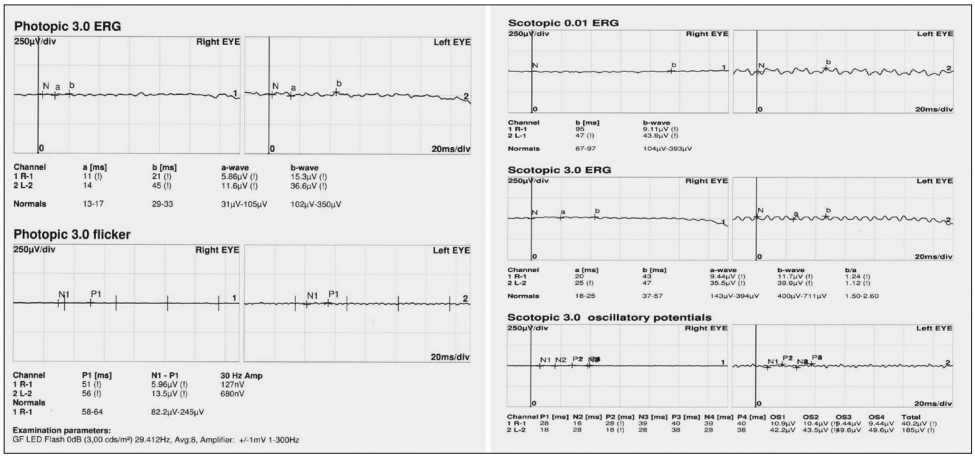

Figure 4: The electrophysiological changes of the LCA patient with novel *RPE65* missense mutation. ERG recordings showed extinguished responses. LCA: Leber congenital amaurosis; ERG: Electroretinogram.

first candidate for gene therapy of this disorder. Most patients in *RPE65* gene therapy exhibited some extent of improvement in visual function without obvious adverse effects.[15-18]

It has been reported that 133 *RPE65* mutations are associated with LCA (HGMD), with the frequency of *RPE65* mutation ranging from 6% to 21%.[3,19] In this study, however, we identified a novel mutation in the 11th exon of *RPE65* (c.1174 A > C), resulting in the substitution of threonine by proline at codon 392 (T392P) in one LCA patient. This novel homozygous missense mutation in *RPE65* was found to be responsible for causing LCA. But in this study cohort of Chinese patients with LCA, only one of the 52 patients recruited was identified to be carrying *RPE65* mutation – a frequency which is much lower than that found in LCA patients in Northwest Europe and the United States.[7] This indicates that *RPE65* mutations may not play a major role in LCA patients in China. However, while estimating the *RPE65* mutation frequency in LCA patients in China may provide useful information for gene therapy of this disease, the LCA patients' cohort in our study may not have been sufficient to estimate an accurate *RPE65* mutation frequency in our LCA patients given that only 1 out of 52 patients carried mutation in *RPE65*. This necessitates further studies with a larger cohort to enhance better understanding of the role of *RPE65* mutations in LCA patients in China.

Acknowledgment

We are grateful to the patients and their family members for their cooperation during this study.

Financial support and sponsorship

This study was supported by grants from the Science and Technology Commission of Beijing Municipality Fund Project (No. Z171100000417039) and National Natural Science Foundation of China (No. 81300789).

Conflicts of interest

There are no conflicts of interest.

References

1. den Hollander AI, Roepman R, Koenekoop RK, Cremers FP. Leber congenital amaurosis: Genes, proteins and disease mechanisms. Prog Retin Eye Res 2008;27:391-419. doi: 10.1016/j.preteyeres.2008.05.003.
2. Nicoletti A, Wong DJ, Kawase K, Gibson LH, Yang-Feng TL, Richards JE, *et al.* Molecular characterization of the human gene encoding an abundant 61 kDa protein specific to the retinal pigment epithelium. Hum Mol Genet 1995;4:641-9. doi: 10.1093/hmg/4.4.641.
3. Morimura H, Fishman GA, Grover SA, Fulton AB, Berson EL, Dryja TP. Mutations in the RPE65 gene in patients with autosomal recessive retinitis pigmentosa or Leber congenital amaurosis. Proc Natl Acad Sci U S A 1998;95:3088-93. doi: 10.1073/pnas.95.6.3088.
4. Perrault I, Rozet JM, Ghazi I, Leowski C, Bonnemaison M, Gerber S, *et al.* Different functional outcome of RetGC1 and RPE65 gene mutations in Leber congenital amaurosis. Am J Hum Genet 1999;64:1225-8. doi: 10.1086/302335.
5. Dharmaraj SR, Silva ER, Pina AL, Li YY, Yang JM, Carter CR, *et al.* Mutational analysis and clinical correlation in Leber congenital amaurosis. Ophthalmic Genet 2000;21:135-50. doi: 10.1076/1381-6810(200009)21:3;1-Z;FT135.
6. Li Y, Wang H, Peng J, Gibbs RA, Lewis RA, Lupski JR, *et al.* Mutation survey of known LCA genes and loci in the Saudi Arabian population. Invest Ophthalmol Vis Sci 2009;50:1336-43. doi: 10.1167/iovs.08-2589.
7. Mamatha G, Srilekha S, Meenakshi S, Kumaramanickavel G. Screening of the RPE65 gene in the Asian Indian patients with Leber congenital amaurosis. Ophthalmic Genet 2008;29:73-8. doi: 10.1080/13816810802008259.
8. Schappert-Kimmijser J, Henkes HE, Van den Bosch J. Amaurosis congenita (Leber). AMA Arch Ophthalmol 1959;61:211-8. doi: 10.1001/archopht.1959.00940090213003.
9. Kaplan J, Bonneau D, Frézal J, Munnich A, Dufier JL. Clinical and genetic heterogeneity in retinitis pigmentosa. Hum Genet 1990;85:635-42. doi: 10.1007/BF00193589.
10. Marlhens F, Bareil C, Griffoin JM, Zrenner E, Amalric P, Eliaou C, *et al.* Mutations in RPE65 cause Leber's congenital amaurosis. Nat Genet 1997;17:139-41. doi: 10.1038/ng1097-139.
11. Lotery AJ, Namperumalsamy P, Jacobson SG, Weleber RG, Fishman GA, Musarella MA, *et al.* Mutation analysis of 3 genes in patients with Leber congenital amaurosis. Arch Ophthalmol 2000;118:538-43. doi: 10.1001/archopht.118.4.538.
12. Xu F, Dong Q, Liu L, Li H, Liang X, Jiang R, *et al.* Novel RPE65 mutations associated with Leber congenital amaurosis in Chinese patients. Mol Vis 2012;18:744-50.
13. Saari JC. Biochemistry of visual pigment regeneration: The Friedenwald lecture. Invest Ophthalmol Vis Sci 2000;41:337-48.
14. Maeda T, Cideciyan AV, Maeda A, Golczak M, Aleman TS, Jacobson SG, *et al.* Loss of cone photoreceptors caused by chromophore depletion is partially prevented by the artificial chromophore pro-drug, 9-cis-retinyl acetate. Hum Mol Genet 2009;18:2277-87. doi: 10.1093/hmg/ddp163.
15. Maguire AM, Simonelli F, Pierce EA, Pugh EN Jr., Mingozzi F, Bennicelli J, *et al.* Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med 2008;358:2240-8. doi: 10.1056/NEJMoa0802315.
16. Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, *et al.* Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med 2008;358:2231-9. doi: 10.1056/NEJMoa0802268.
17. Hauswirth WW, Aleman TS, Kaushal S, Cideciyan AV, Schwartz SB, Wang L, *et al.* Treatment of Leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: Short-term results of a phase I trial. Hum Gene Ther 2008;19:979-90. doi: 10.1089/hum.2008.107.
18. Simonelli F, Ziviello C, Testa F, Rossi S, Fazzi E, Bianchi PE, *et al.* Clinical and molecular genetics of Leber's congenital amaurosis: A multicenter study of Italian patients. Invest Ophthalmol Vis Sci 2007;48:4284-90. doi: 10.1167/iovs.07-0068.
19. Yzer S, Leroy BP, De Baere E, de Ravel TJ, Zonneveld MN, Voesenek K, *et al.* Microarray-based mutation detection and phenotypic characterization of patients with Leber congenital amaurosis. Invest Ophthalmol Vis Sci 2006;47:1167-76. doi: 10.1167/iovs.05-0848.