• Letter to the Editor •

Digenic heterozygous mutations in *EYS/LRP5* in a Chinese family with retinitis pigmentosa

Feng-Juan Gao[1], Sheng-Hai Zhang[1,2,3], Jun-Yi Chen[1], Ge-Zhi Xu[1,2,3], Ji-Hong Wu[1,2,3]

[1]Eye & ENT Hospital, State Key Laboratory of Medical Neurobiology, Institutes of Brain Science and Collaborative Innovation Center for Brain Science, Shanghai Medical College, Fudan University, Shanghai 200032, China
[2]Shanghai Key Laboratory of Visual Impairment and Restoration, Shanghai 200032, China
[3]Key Laboratory of Myopia, Ministry of Health, Fudan University, Shanghai 200032, China
Correspondence to: Ji-Hong Wu. Eye & ENT Hospital, Fudan University, 83# Fenyang road, Shanghai 200032, China. jihongwu@fudan.edu.cn
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Dear Editor,

I am Dr. Ji-Hong Wu, from the Department of Ophthalmology, Eye & ENT Hospital of Fudan University, China. I write to present a case report of retinitis pigmentosa (RP) caused by novel digenic heterozygous mutations in a Chinese family.

RP, the most frequently described inherited retinal dystrophies (IRD), shows a prevalence of 1/3500 to 1/5000 worldwide, but statistics from the Beijing Eye Research Center show that the incidence of RP in China is approximately 1 in 1000[1]. RP commences with progressive night blindness, followed by gradual loss of peripheral visual fields (VF) and complete blindness in the later stage[2]. RP can be transmitted *via* autosomal dominant (24%), autosomal recessive (41%), and X-linked (22%)[3]. The remaining 12% of cases are presumed to cause by non-genetic factors, non-Mendelian inheritance (for example, mitochondrial or de novo mutations) or complex inheritance (digenic or polygenic inheritance) [4].

To date, more than 60 genes have been associated with RP. *EYS* is considered to be one of the most prevalent mutated genes worldwide[5]. The *EYS* gene contains 43 exons and spanning over 2000 bp within the RP25 locus (6q12). *EYS* encodes a protein of 3165 amino acids, which is localized to the outer segment of the photoreceptor cell layer[6]. *EYS* mutations demonstrate great genotypic and phenotypic varieties, and are one of the primary major gene responsible for recessive RP[7]. The low-density lipoprotein (LDL)-related receptor-5 (*LRP5*), a member of the LDL receptor superfamily, is a co-receptor for Wnt ligands involved in the wingless (Wnt) signaling pathway, which is essential for the development of vascular endothelial cells, Müller cells and retinal interneurons[8]. Loss-of-function mutations in *LRP5* can cause familial exudative vitreoretinopathy (FEVR) in humans in the way of recessive RP[9], but no mutations have been identified in RP.

Diverse diagnostic strategies are used in RP cases. Custom-designed re-sequencing microarrays is an effective alternative for the detection of novel mutations, although this approach is limited to a known set of genes. Sanger sequencing is an indispensable and the most reliable method for identifying causative gene mutations, although it is not affordable due to the great genetic heterogeneity of RP. However, most of these limitations can now be overcome with next-generation sequencing (NGS), which has become widely used and facilitated the discovery of many causative genes and gene variants of complex traits[10]. In this study, we conducted a high-throughput sequence capture microarray with 99.67% coverage of all exons and combined with NGS to identify the possible causal genes in a Chinese family with RP, in which the cause of the disease had not been determined yet.

Our study involved a Chinese family consisting of the proband and his unaffected parents. The proband, a 30-year-old man, received a diagnosis of RP on the basis of clinical symptoms, such as decreased vision, night blindness, red-green blindness and the results of a comprehensive ocular examination, as described elsewhere[11]. Fundus examination revealed attenuated vessels and bone spicule-shaped pigment deposits in the peripheral retina in both eyes (Figure 1); spectral-domain optical coherence tomography (SD-OCT) line scans of both eyes through the central maculae showed a profound loss of photoreceptor layer structure in the retina. The remaining photoreceptors and the transitional zone were only seen in the macular zone (Figure 1); No detectable cone or rod responses were recorded by electroretinography (ERG) in the patient (Figure 2). All of the retinal phenotypes of the patient were typical for RP without any other ocular abnormalities. His parents reported no major issues with vision, and there was no family history of ocular or systemic diseases.

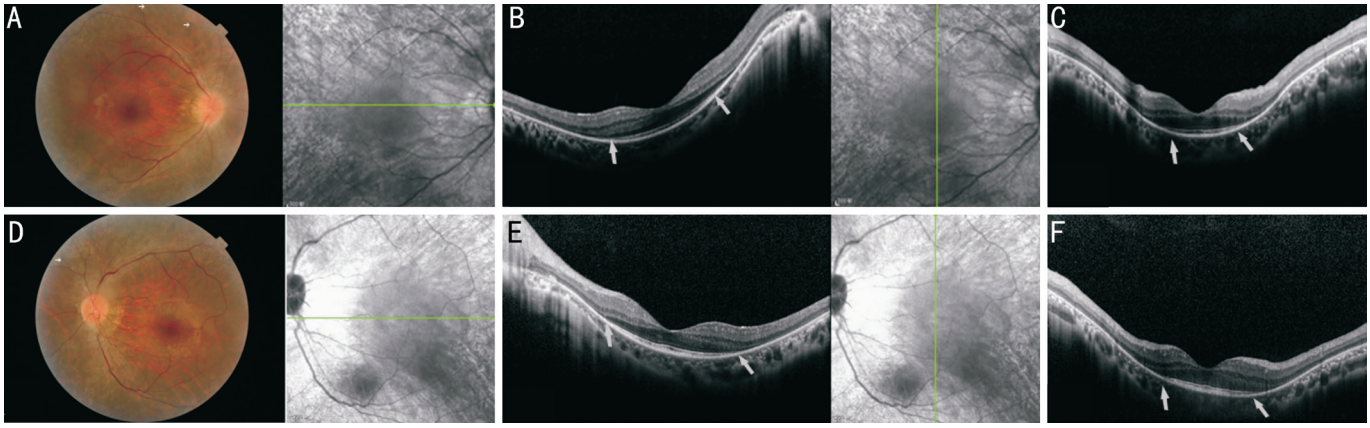

Figure 1 Fundus photographs and SD-OCT scans of the right and left eyes of the proband Fundus photography (A: right eye; D: left eye) in the proband exhibited attenuated vessels and bone spicule-shaped pigment deposits in the peripheral retina (marked by white arrow) in both eyes. SD-OCT scans along the horizontal (B: right eye; E: left eye) and vertical (C: right eye; F: left eye) meridian of the central retina highlighted the remaining photoreceptors and the transitional zone (marked by white arrow heads).

Table 1 Overview of data production

Gene	NCBI reference sequence	Nucleotide change	Amino acid substitution	Chromosomal location	Gene subregion	Allele status
EYS	NM_001142800	c.7723+1G>A	-	Chr6:6449797	IN39	Het
LRP5	NM_002335	c.3361 A>G	p.Asn1121Asp	Chr11:68192694	EX15/CDS1	Het

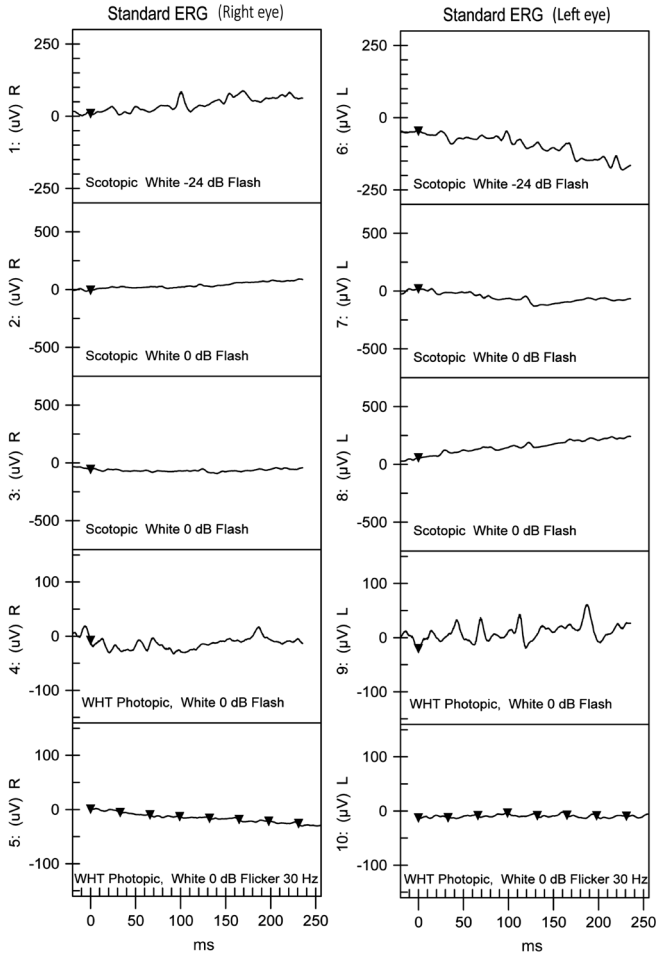

Figure 2 ERG results of the right and left eyes of the proband. Both dark-adapted and light-adapted responses were non-detectable.

Genomic DNA from the family was extracted from the peripheral blood using a standard method[12]. The index patient was analyzed firs. We performed a targeted NGS approach on the patient. The NGS strategy included 129 target genes,

Table 2 Mutations identified in the present study

No. of target genes	129
Target gene length (bp)	495 953
Coverage of target region	99.67%
Mean depth of target region (X)	264.26
Fraction of target covered ≥30 X (%)	96.80%

Het: Heterozygous.

spanning a length of 495 953 bp, with 191.5X depth and 99.65% coverage of the targeted region (Table 1). Fifteen heterozygous mutations and two homozygous mutations, including five novel and eleven known mutations, were identified. After subjecting the results to a pipeline of database filtering, including the dbSNP137, HapMap Project, 1000 Genome Project, YH database and Exome Variant Server databases, a novel nonsense mutation c.7723+1G>A in the *EYS* gene and a formerly reported missense mutation c.3361A>G in the *LRP5* gene[9] were identified as potentially causative mutations for RP (Figure 3, Table 2). Sanger sequencing for these two mutations in his parents showed that his mother carried the *EYS* c.7723+1G>A mutation in the heterozygous state, and his father carried the *LRP5* c.3361 A>G mutation in the heterozygous state.

The advent of NGS has significantly increased the possibilities of identification of many rare disease genes, including genes for retinal dystrophies. NGS is a high-throughput tool which is capable of sequencing large gene pools efficiently and precisely, so it provides large data sets. Therefore, NGS has become a powerful and cogent tool for elucidating thorough mutation profiles for heterogeneous diseases. The clinical and genetic complexity of RP makes the accurate diagnosis

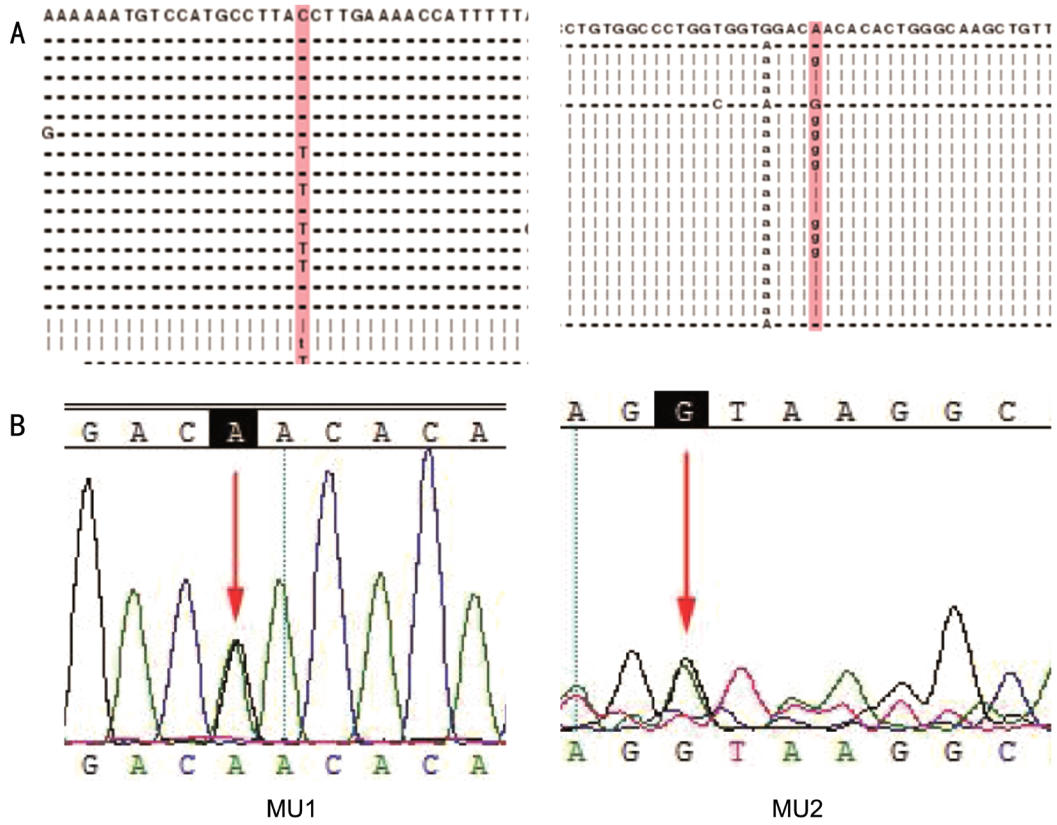

Figure 3 Mutations identified in the *EYS* and *LRP5* genes A: Protein alignment for the mutations identified in *EYS* (left) and *LRP5* (right) gene; B: Sequencing results of the novel mutation in the *EYE* gene (MU1) and *LRP5* gene (MU2). Arrows indicate the position of the mutated nucleotide.

of some cases possible only through NGS approaches. In this study, we comprehensively screened 129 genes involved in common inherited nonsyndromic eye diseases and successfully identified 2 potentially causative mutations for RP: *EYS* c.7723+1G>A and *LRP5* c.3361 A>G.

The types of *EYS* mutations identified in RP include missense, nonsense, microdeletions and insertions, 5'UTR variations, and copy number variations, such as midsize genomic rearrangements[13]. The onset ages of RP caused by *EYS* varied greatly from 6- to 62-year-old[14]. In the present study, we detected a novel splicing mutation of *EYS* c.7723+1G>A in the proband in a heterozygous state. The potential pathogenicity of the filtered variants was then interpreted according to the existing and proposed American College of Medical Genetics and Genomics guidelines. Result shows that EYS c.7723+1G>A is splice site mutation, which may change the splicing way of the RNA precursor, resulting in abnormal protein coding. However, his unaffected mother is also in a heterozygous state, implying that heterozygous mutation of EYS c.7723+1G>A alone may not be sufficient to cause RP.

Mutations in the gene *LRP5* have been shown to be responsible for both FEVR and osteoporosis-pseudoglioma syndrome, a disease that is characterized by blindness and loss of bone mass[15]. Heterozygous mutation of *LRP5* c.3361 A>G has been reported in FEVR but not in RP. However, this previous study only tested the coding exons and adjacent intronic regions of the *FZD4* and *LRP5* genes. Thus, mutations in the intronic or regulatory regions could not be detected, and we cannot rule out the possibility of the existence of other mutations at work. In our study, we found that the mutation was present in both the proband and his unaffected father in heterozygous status, suggesting that single heterozygous mutation of *LRP5* c.3361 A>G might not be sufficient to cause RP or FEVR.

Our data suggest a possible digenic form of inheritance for RP, whereby the co-existence of *EYS* c.7723+1G>A and *LRP5* c.3361A>G heterozygous mutations can result in RP. Because *EYS* c.7723+1G>A is a splicing mutation and *LRP5* c.3361A>G is missense mutation, we hypothesize this result can be explained by the fact that the *LRP5* c.3361A>G mutation affects only part of the mRNA, and the normal amount of *LRP5* protein is sufficient to sustain proper functioning of the cells. The *EYS* splicing mutation may affect pre-mRNA splicing and mRNA stability and translation efficiency; as a result, normal *LRP5* protein is decreased, whereas abnormal protein accumulates and eventually leads to RP. However, there may be other unknown mechanisms responsible for the phenotype observed. This study may provide for the first time evidence for the digenic or polygenic nature of this disease, which further extended our current understanding of the genetic basis of RP, although more samples are needed to further understand the mutation spectrum of RP.

In summary, this is the first report that digenic heterozygous mutations of *EYS* c.7723+1G>A and *LRP5* c.3361A>G can cause RP in a Chinese individual. These results enhance our current understanding of the genetic basis of RP and provide helpful clues for designing future studies to further investigate genetic factors associated with familial RP.

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