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Identification of novel variants in retinitis pigmentosa genes by whole-exome sequencing

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SUMMARY

OBJECTIVE: Retinitis pigmentosa is an inherited degenerative disorder causing severe retinal dystrophy and visual impairment, mainly with onset in the first or second decades. The next-generation sequencing has become an efficient tool to identify disease-causing mutations in retinitis pigmentosa. The aim of this retrospective study was to investigate novel gene variants and evaluate the utility of whole-exome sequencing in patients with retinitis pigmentosa.

METHODS: The medical records of 20 patients with retinitis pigmentosa at Eskişehir City Hospital between September 2019 and February 2022 were analyzed retrospectively. Peripheral venous blood was obtained, followed by the extraction of genomic DNAs. The medical and ophthalmic histories were collected, and ophthalmological examinations were performed. Whole-exome sequencing was performed to determine the genetic etiology of the patients.

RESULTS: The proportion of genetically solved cases was 75% (15/20) in the patients with retinitis pigmentosa. Molecular genetic testing identified 13 biallelic and 4 monoallelic mutations in known retinitis pigmentosa genes, including 11 novel variants. According to ***in silico*** prediction tools, nine variants were predicted as pathogenic or possibly pathogenic. We identified six previously reported mutations to be associated with retinitis pigmentosa. The age of onset of the patients ranged from 3 to 19, with a mean age of onset of 11.6. All patients had a loss of central vision.

CONCLUSION: As the first study of the application of whole-exome sequencing among patients with retinitis pigmentosa in a Turkish cohort, our results may contribute to the characterization of the spectrum of variants related to retinitis pigmentosa in the Turkish population. Future population-based studies will enable us to reveal the detailed genetic epidemiology of retinitis pigmentosa.

KEYWORDS: Night blindness. Frameshift mutation. Mutation. Retinitis pigmentosa. Sequence analysis.

INTRODUCTION

Retinitis pigmentosa (RP) is a group of genetic disorders resulting in inherited blindness due to the degeneration of rod and cone photoreceptors[1]. RP is associated with significant genotypic and phenotypic heterogeneity, with more than 89 genes causing RP reported so far[2,3]. Despite this heterogeneity, RP patients have some common clinical features: progressive loss of photoreceptors, typically involving the rod system. The characteristic phenotype includes retinal bone-spicule pigmentation, pallor of the optic disk, and attenuation of the retinal vessel[1-3]. It is estimated to affect about 1 in 3,000 to 1 in 4,000 people worldwide[4]. The genetic condition may be autosomal dominant RP (15–25%), autosomal recessive (31–41%), or X-linked recessive trait (12–22%) Moreover, approximately 50% of RP cases are sporadic[5]. In recent years, the application of next-generation sequencing (NGS), mostly as targeted exome sequencing (TES) and whole-exome sequencing (WES), has greatly increased the genetic diagnosis rates of different forms of RP[6-9]. The diagnosis rate of TES in RP patients ranges from about 30 to 65%[10-12]. Despite the large number of disease-related genes identified, the majority of patients with RP do not appear to have any genetic defects in all known genes[13,14]. Nevertheless, WES is useful for identifying novel disease-related genes, albeit at a higher cost than TES[15,16]. As a result of the use of new-generation genetic technologies, the rapidly increasing new information leads both to illuminate the genetic etiology and to define new clinical entities with diagnosis and treatment options[14-16]. This retrospective study aims to describe the phenotype and genotype of Turkish patients with RP. This is the first comprehensive molecular diagnosis of a Turkish RP patient cohort using WES. Here, we report the genetic and ophthalmological findings in 20 Turkish patients with RP with 17 variants, including 11 novel mutations in RP genes.

METHODS

This retrospective single-center study included the subjects who were investigated at the Department of Ophthalmology.

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Between September 2019 and February 2022, the patients were evaluated by an ophthalmologist and clinically diagnosed with RP. The patient's age, gender, age of onset, family history, clinical, and ocular examination findings were noted. The diagnosis of RP was based on the detection of topographically limited retinal abnormalities consistent with corresponding sectorial visual field defects. Best-corrected visual acuity (BCVA), fundus color pictures as well as fundus autofluorescence (FAF), spectral-domain optical coherence tomography (SD-OCT), full-field electroretinography (ERG), color vision, and fundus photography were retrospectively collected and analyzed. The study was approved by the Ethics Committee of the Eskişehir Osmangazi Medical Faculty (Protocol number: 2022-111, Decision date/number: April 24, 2022/42). This study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all the patients. Prior to genetic testing, a diagnosis of RP was made based on a history of structural retinal changes and/or visual field defects consistent with the disease. Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. After the clinical diagnosis of RP, we proceeded with next-generation sequencing of the whole exome in the probands, performing the TWIST© Human Core Exome® kit with 97.11% of targeted regions covered at ≥20×. Variants were filtered against dbNSFP v2.0, dbSNP v137, and population databases including the Genome Aggregation Database (gnomAD), the Exome Aggregation Consortium (ExAC), and the 1000 Genomes Project. All variants with a MAF ≤0.01 were evaluated and classified as pathogenic (P), likely pathogenic (LP), variants of uncertain significance (VUS), likely benign (LB), and benign (B) according to the criteria and guidelines of the American College of Medical Genetics and Genomics (ACMG). The variants identified as pathogenic in ClinVar and/or Human Genome Mutation Database were considered to explain the phenotype. Deleterious effect prediction of the variants used multiple algorithms, including Sorting Intolerant From Tolerant (SIFT), Polymorphism Phenotyping v2 (PolyPhen2), and MutationTaster. The statistical analyses were done using the SPSS 15.0 software. In this analysis, clinical data were expressed in percentages.

RESULTS

In total, 20 patients from 18 families with RP were included in this study. There was a male preponderance, forming 80% of the total cases (16/20). The mean age of the patients was 38.46 years (a range of 19–57). The mean age at disease onset was 11.6 years (a range of 3–19). Whole-exome sequencing revealed one or more RP disease-causing alleles in 15/20 (75%) of the patients. In 5 of 20 cases (25%), a genetic diagnosis was not achieved. Table 1 shows demographic characteristics, age at

Table 1. Clinical and demographic characteristics in 15 patients with retinitis pigmentosa.

Patients	Sex, age (years)	Age of onset	Consanguinity in parents	Fundoscopy	Gene	Genetic diagnosis-inheritance
Case 1	Male, 54	14	Yes	ONP, ARA, PBSL	ARL2BP	RP 82 (AR)
Case 2	Male, 29	5	Yes	ARA, CA with foveal sparing, ONP	PCARE	RP 54 (AR)
Case 3	Male, 32	7	Yes	ONP, GRC, ARA	PCARE	RP 54 (AR)
Case 4	Male, 31	14	No	ARA, CA with foveal sparing, PBSL	CERKL	RP 26 (AR)
Case 5	Female, 30	4	Yes	GRC, ONP, PBSL	NR2E3	RP 37 (AR)
Case 6	Female, 45	9	Yes	PF, ARA, PBSL	EYS/ RP1	RP 25 (AR) / RP 1 (AD/AR) (digenic inheritance)
Case 7	Female, 57	16	No	PF, ARA, ONP, PBSL	CERKL	RP 26 (AR)
Case 8	Female, 54	6	Yes	CA, ONP, ARA, PBSL	CRB1	RP 12 (AR)
Case 9	Female, 19	3	Yes	CA with foveal sparing, ARA, PBSL	ABCA4	RP 19 (AR)
Case 10	Male, 19	3	Yes	CA, PF, ARA	ABCA4	RP 19 (AR)
Case 11	Male, 37	17	No	CA, ARA, PBSL	EYS	RP 25 (AR)
Case 12	Male, 40	4	Yes	ONP, GRC, ARA, PBSL	MERTK	RP 38 (AR)
Case 13	Male, 44	16	Yes	ONP, GRC, ARA, PBSL	USH2A	RP 39 (AR)
Case 14	Male, 52	19	No	CA with foveal sparing, PF, PBSL	RPGR	RP 3 (XR)
Case 15	Male, 32	4	Yes	CA, ONP, ARA, PBSL	RPE65	RP 20 (AR)

ARA: attenuated retinal arteries; GRC: gray retinal color; ONP: optic nerve pale; PBSL: pigment bone spicule-like; CA: central atrophy; PF: pale fundus.

onset of disease, clinical findings, and the diagnoses of patients with mutations detected in genetic test results. A total of 17 variants were found that could explain the RP phenotype. Among these, 11 were novel variants (4 missense, 3 nonsense, 3 frameshift mutations, and 1 intronic variant). Of these 15 probands, 12 were homozygous for causative variants (80%). Two probands had compound heterozygous mutations in recessive-RP-related genes (*EYS/RP1* and *USH2A*), and one patient had hemizygous for an X-linked gene (RPGR) (Table 2).

The *in silico* protein prediction results of the novel mutations are presented in Tables 2 and 3. Pathogenicity was interpreted in accordance with MutationTaster, PolyPhen-2, and SIFT. According to the prediction tools, one variant (*USH2A*: c.4348G>A) was predicted as of uncertain significance and one variant (*RP1*: c.2386G>A) was predicted as tolerable/benign. Nine of the 11 novel variants were predicted as pathogenic or likely pathogenic (81%) (Table 3).

A mutation in the *RPGR* gene was detected in only one patient with X-linked RP (Tables 1 and 2). In 14 patients with autosomal recessive RP, several mutations were revealed in *ARL2BP*, *PCARE*, *EYS/RP1* (biallelic variants), *CRB1*, *ABCA4*, *EYS*, *CERKL*, *MERTK*, *RPE65*, *USH2A*, and *NR2E3* (compound heterozygous) (Table 2). We also identified six previously reported mutations related to RP (*NR2E3*, *CRB1*, *ABCA4*, and *EYS*) (Table 2). The presence of attenuated retinal arteries was detected in 13 patients (86.6%), bone spicule pigmentation in 12 patients (80%), and pallor of the optic nerve or fundus in 11 patients (73.3%) of genetically diagnosed patients (Table 1).

DISCUSSION

The present study recruited 20 patients who had received a clinical diagnosis of RP and had them undergo whole-exome sequencing with the aim of identifying pathogenic variants. A genetic diagnosis was possible in 15 cases in this study. To the best of authors' knowledge, this is the first report to evaluate the diagnosis rate and causative genes among Turkish patients with RP using whole-exome sequencing. Previous results showed that the detection rate of genetic diagnosis in patients with RP by targeted exome sequencing ranged from 30 to 65%[17-19]. We have identified 17 gene variants out of 15 Turkish patients with RP; of these, 11 (64.7%) were novel. The rate found in our study was found to be compatible with recent

Table 2. The disease-associated variants identified in 15 patients.

Patient	Family	Gene	Zygosity	Allele 1	Publication	Allele 2	Publication
P1	F1	ARL2BP	Homozygous	c.403C>T, p.Arg135Ter	Novel	c.403C>T, p.Arg135Ter	Novel
P2	F2	PCARE	Homozygous	c.1541delC, p.Pro514HisfsTer27	Novel	c.1541delC, p.Pro514HisfsTer27	Novel
P3	F2	PCARE	Homozygous	c.1541delC, p.Pro514HisfsTer2	Novel	c.1541delC, p.Pro514HisfsTer2	Novel
P4	F3	CERKL	Homozygous	c.1566_1567insCCAA-GACTTATCAGTCTTTA, p. Gly523ProfsTer14	Novel	c.1566_1567insCCAA-GACTTATCAGTCTTTA, p. Gly523ProfsTer14	Novel
P5	F4	NR2E3	Compound heterozygous	c.309C>A, p.Cys103Ter	Reported	c.227G>A, p.Arg76Gln	Reported
P6	F5	EYS/ RP1	Biallelic (digenic) Heterozygous	EYS: c. 2949delC, p.Tyr983Ter	Novel	RP1:c.2386G>A, p.Gly796Ser	Novel
P7	F6	CERKL	Homozygous	c.271G>T, p.Glu91Ter	Novel	c.271G>T, p.Glu91Ter	Novel
P8	F7	CRB1	Homozygous	c.2230C>T, p.Arg744Ter	Reported	c.2230C>T,p.Arg744Ter	Reported
P9	F8	ABCA4	Homozygous	c.1804C>T, p.Arg602Trp	Reported	c.1804C>T, p.Arg602Trp	reported
P10	F8	ABCA4	Homozygous	c.1804C>T, p.Arg602Trp	Reported	c.1804C>T, p.Arg602Trp	reported
P11	F9	EYS	Homozygous	c.8793_8796delATCA, p.Gln2931HisfsTer43	Clinvar	c.8793_8796delATCA, p.Gln2931HisfsTer43	Clinvar
P12	F10	MERTK	Homozygous	c.1604+5G>A	Novel	c.1604+5G>A	Novel
P13	F11	USH2A	Compound heterozygous	c.5386T>C, p.Cys1796Arg	Novel	c.4348G>A, p.Val1450Ile	Novel
P14	F12	RPGR	Hemizygous	c.2234_2237delGAGA, p.Arg745LysfsTer69	Novel	Not determineed	(-)
P15	F13	RPE65	Homozygous	c.314C>T, p.Thr105Ile	Novel	c.314C>T, p.Thr105Ile	Novel

Table 3. Pathogenicity predictions for the 11 novel variants in RP genes reported in the present study.

Gene	Nucleotide change	Protein change	MutationTaster	PolyPhen2	SIFT
ARL2BP	c.403C>T (nonsense variant)	p.Arg135Ter	Disease causing	Damaging	Pathogenic
PCARE	c.1541delC (frameshift variant)	p.Pro514HisfsTer27	Disease causing	–	Pathogenic
CERKL	c.1566_1567insCCAAGACTTATCAGTCTTTA (frameshift variant)	p.Gly523ProfsTer14	Disease causing	–	Pathogenic
EYS	c.2949delC (nonsense variant)	p.Tyr983Ter	Likely Pathogenic	Probably damaging	Likely pathogenic
RP1	c.2386G>A (missense variant)	p.Gly796Ser	Polymorphism	Likely benign	Tolerated
CERKL	c.271G>T (nonsense variant)	p.Glu91Ter	Disease causing	Damaging	Pathogenic
MERTK	c.1604+5G>A (intronic variant)	-	Likely Pathogenic	Probably damaging	Likely pathogenic
USH2A	c.5386T>C (missense variant)	p.Cys1796Arg	Disease causing	Damaging	Pathogenic
USH2A	c.4348G>A (missense variant)	p.Val1450Ile	Uncertain Significance	Uncertain Significance	Uncertain significance
RPGR	c.2234_2237delGAGA (frameshift variant)	p.Arg745LysfsTer69	Disease causing	-	Pathogenic
RPE65	c.314C>T (missense variant)	p.Thr105Ile	Likely Pathogenic	Probably damaging	Likely pathogenic

SIFT: sorting intolerant from tolerant; PolyPhen2: polymorphism phenotyping v2.

studies reporting novel gene mutation rates ranging from 62 to 68%[20,21]. Variants in four genes (*NR2E3*, *CRB1*, *ABCA4*, and *EYS*) have been reported to be responsible for RP12 (AR), RP19 (AR), RP25 (AR), and RP37 (AR), respectively. Based on the genetic findings, inheritance turned out to be autosomal recessive in 93.3% (14 out of 15) and X-linked in 6.7% (1 out of 15) of patients. The AR RP (93.3%) was detected in the majority of the patients in our study. No proband was found with AD RP in this study.

The mutations in *ARL2BP* are a known cause of RP82 (AR)[22]. To the best of authors' knowledge, approximately 10 cases have been reported with RP82 due to a homozygous mutation in *ARL2BP* in the medical literature[23]. Herein, we report the 11th patient with RP82 in the world and the first patient from Turkey.

The *EYS* mutations can cause RP25 (AR). The *RP1* mutations have been associated with RP1 (AR/AD). The segregation analysis showed that the parents were carriers of this variant[24]. Segregation analyses pointed toward a digenic inheritance. Gao et al. reported the co-existence of *EYS* c.7723+1G>A and *LRP5* c.3361A>G heterozygous mutations in a patient with RP[25]. Herein, this is the first study in which *EYS* and *RP1* gene variants were found together in an RP patient with a digenic biallelic disease.

In this study, we present a comprehensive clinical and genetic evaluation of individuals with RP. To the best of authors' knowledge, this is the first retrospective study that includes a cohort of subjects of Turkish origin with RP. The genetic results of the present study conducted with a Turkish population showed that most of the patients were predominantly compatible with the diagnosis of AR RP (93.3%). The rate of genetically resolved cases was 75% in our study. The overall diagnostic yield of targeted gene sequencing is 55–65%[11].

Herein, we also identified 11 novel variants in RP-related genes. These results will contribute to expanding the mutational spectrum of RP genes. Approximately 81% (9/11) of the identified novel variants are pathogenic or likely pathogenic. The rate in this study is higher than that observed in similar studies from Europe and the Far East, where approximately 45 and 63% of the pathogenic alleles were novel[18,19]. These results confirm the utility of WES as a powerful method for mutation identification in the diagnosis of RP.

The limitations of our study are represented by the relatively small sample size, the retrospective nature of the study, and, as explained above, the fact that we did not use the same section as a reference for all follow-up examinations.

CONCLUSION

The WES analysis may help to provide a more accurate clinical diagnosis in the detection of genetic diseases with high heterogeneity, such as RP. Meanwhile, we are highlighting the importance of comprehensive NGS-based tests in screening genetically unresolved cases for known RP genes as well as other retinal disease genes. Our current knowledge of the mutation spectrum underlying RP in other populations is limited, as most studies of RP have been conducted with patients of European origin. Identification of the molecular diagnosis of RP patients in different populations will expand the global spectrum of RP-associated gene mutations.

AUTHOR CONTRIBUTIONS

AK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing.

İAÖ: Data curation, Investigation, Writing – review & editing.

NDU: Data curation, Investigation, Writing – review & editing.

BP: Investigation, Methodology, Writing – review & editing.

REFERENCES

1. Verbakel SK, Huet RAC, Boon CJF, Hollander AI, Collin RWJ, Klaver CCW, et al. Non-syndromic retinitis pigmentosa. Prog Retin Eye Res. 2018;66:157-86. https://doi.org/10.1016/j.preteyeres.2018.03.005
2. Dias MF, Joo K, Kemp JA, Fialho SL, Silva Cunha A, Woo SJ, et al. Molecular genetics and emerging therapies for retinitis pigmentosa: basic research and clinical perspectives. Prog Retin Eye Res. 2018;63:107-31. https://doi.org/10.1016/j.preteyeres.2017.10.004
3. Comander J, Weigel-DiFranco C, Maher M, Place E, Wan A, Harper S, et al. The genetic basis of pericentral retinitis pigmentosa-A form of mild retinitis pigmentosa. Genes (Basel). 2017;8(10):256. https://doi.org/10.3390/genes8100256
4. Birtel J, Gliem M, Mangold E, Müller PL, Holz FG, Neuhaus C, et al. Next-generation sequencing identifies unexpected genotype-phenotype correlations in patients with retinitis pigmentosa. PLoS One. 2018;13(12):e0207958. https://doi.org/10.1371/journal.pone.0207958
5. Lee SH, Yu HG, Seo JM, Moon SW, Moon JW, Kim SJ, et al. Hereditary and clinical features of retinitis pigmentosa in Koreans. J Korean Med Sci. 2010;25(6):918-23. https://doi.org/10.3346/jkms.2010.25.6.918
6. Huang L, Zhang Q, Huang X, Qu C, Ma S, Mao Y, et al. Mutation screening in genes known to be responsible for retinitis pigmentosa in 98 small han Chinese families. Sci Rep. 2017;7(1):1948. https://doi.org/10.1038/s41598-017-00963-6
7. Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. N Engl J Med. 2013;369(16):1502-11. https://doi.org/10.1056/NEJMoa1306555
8. Yoon CK, Kim NK, Joung JG, Shin JY, Park JH, Eum HH, et al. The diagnostic application of targeted re-sequencing in Korean patients with retinitis pigmentosa. BMC Genomics. 2015;16(1):515. https://doi.org/10.1186/s12864-015-1723-x
9. Bravo-Gil N, González-Del Pozo M, Martín-Sánchez M, Méndez-Vidal C, Rodríguez-de la Rúa E, Borrego S, et al. Unravelling the genetic basis of simplex retinitis pigmentosa cases. Sci Rep. 2017;7:41937. https://doi.org/10.1038/srep41937
10. Chang S, Vaccarella L, Olatunji S, Cebulla C, Christoforidis J. Diagnostic challenges in retinitis pigmentosa: genotypic multiplicity and phenotypic variability. Curr Genomics. 2011;12(4):267-75. https://doi.org/10.2174/138920211795860116
11. Chizzolini M, Galan A, Milan E, Sebastiani A, Costagliola C, Parmeggiani F. Good epidemiologic practice in retinitis pigmentosa: from phenotyping to biobanking. Curr Genomics. 2011;12(4):260-6. https://doi.org/10.2174/138920211795860071
12. Teare MD, Santibañez Koref MF. Linkage analysis and the study of Mendelian disease in the era of whole exome and genome sequencing. Brief Funct Genomics. 2014;13(5):378-83. https://doi.org/10.1093/bfgp/elu024
13. Daiger SP, Sullivan LS, Bowne SJ. Genes and mutations causing retinitis pigmentosa. Clin Genet. 2013;84(2):132-41. https://doi.org/10.1111/cge.12203
14. Huang XF, Huang F, Wu KC, Wu J, Chen J, Pang CP, et al. Genotype-phenotype correlation and mutation spectrum in a large cohort of patients with inherited retinal dystrophy revealed by next-generation sequencing. Genet Med. 2015;17(4):271-8. https://doi.org/10.1038/gim.2014.138
15. Chen L, Wang N, Lai M, Hou F, He J, Fan X, et al. Clinical and genetic investigations in Chinese families with retinitis pigmentosa. Exp Biol Med (Maywood). 2022;247(12):1030-8. https://doi.org/10.1177/15353702221085711
16. Nishiguchi KM, Tearle RG, Liu YP, Oh EC, Miyake N, Benaglio P, et al. Whole genome sequencing in patients with retinitis pigmentosa reveals pathogenic DNA structural changes and NEK2 as a new disease gene. Proc Natl Acad Sci USA. 2013;110(40):16139-44. https://doi.org/10.1073/pnas.1308243110
17. Neveling K, Collin RW, Gilissen C, Huet RA, Visser L, Kwint MP, et al. Next-generation genetic testing for retinitis pigmentosa. Hum Mutat. 2012;33(6):963-72. https://doi.org/10.1002/humu.22045
18. Wang F, Wang H, Tuan HF, Nguyen DH, Sun V, Keser V, et al. Next generation sequencing-based molecular diagnosis of retinitis pigmentosa: identification of a novel genotype-phenotype correlation and clinical refinements. Hum Genet. 2014;133(3):331-45. https://doi.org/10.1007/s00439-013-1381-5
19. Wang J, Zhang VW, Feng Y, Tian X, Li FY, Truong C, et al. Dependable and efficient clinical utility of target capture-based deep sequencing in molecular diagnosis of retinitis pigmentosa. Invest Ophthalmol Vis Sci. 2014;55(10):6213-23. https://doi.org/10.1167/iovs.14-14936
20. Xu Y, Guan L, Shen T, Zhang J, Xiao X, Jiang H, et al. Mutations of 60 known causative genes in 157 families with retinitis pigmentosa based on exome sequencing. Hum Genet. 2014;133(10):1255-71. https://doi.org/10.1007/s00439-014-1460-2
21. Eisenberger T, Neuhaus C, Khan AO, Decker C, Preising MN, Friedburg C, et al. Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: the example of retinal dystrophies. PLoS One. 2013;8(11):e78496. https://doi.org/10.1371/journal.pone.0078496
22. Davidson AE, Schwarz N, Zelinger L, Stern-Schneider G, Shoemark A, Spitzbarth B, et al. Mutations in ARL2BP, encoding ADP-ribosylation-factor-like 2 binding protein, cause autosomal-recessive retinitis pigmentosa. Am J Hum Genet. 2013;93(2):321-9. https://doi.org/10.1016/j.ajhg.2013.06.003
23. Audo I, El Shamieh S, Méjécase C, Michiels C, Demontant V, Antonio A, et al. ARL2BP mutations account for 0.1% of autosomal recessive rod-cone dystrophies with the report of a novel splice variant. Clin Genet. 2017;92(1):109-11. https://doi.org/10.1111/cge.12909
24. Gao FJ, Zhang SH, Chen JY, Xu GZ, Wu JH. Digenic heterozygous mutations in EYS/LRP5 in a Chinese family with retinitis pigmentosa. Int J Ophthalmol. 2017;10(2):325-8. https://doi.org/10.18240/ijo.2017.02.25
25. Fu Q, Wang F, Wang H, Xu F, Zaneveld JE, Ren H, et al. Next-generation sequencing-based molecular diagnosis of a Chinese patient cohort with autosomal recessive retinitis pigmentosa. Invest Ophthalmol Vis Sci. 2013;54(6):4158-66. https://doi.org/10.1167/iovs.13-11672