JOURNAL OF
CONTEMPORARY MEDICINE

DOI:10.16899/jcm.1131536
J Contemp Med 2022;12(5):749-756

Original Article / Orijinal Araştırma

Genetic and Clinical Evaluation of Retinitis Pigmentosa

Retinitis Pigmentosa'nın Genetik ve Klinik Değerlendirilmesi

Özgür Eroğul[1], Muhsin Elmas[2], Mustafa Doğan[1], Hamidu Hamisi Gobeka[1], Ayça Nur Demir[3], Leyla Eryiğit Eroğul[4]

[1]Afyonkarahisar Health Science University, Faculty of Medicine, Department of Ophthalmoloy, Afyonkarahisar, Turkey
[2]Afyonkarahisar Health Science University, Faculty of Medicine, Department of Genetic, Afyonkarahisar, Turkey
[3]Afyonkarahisar Health Sciences University Faculty of Medicine, Afyonkarahisar, Turkey
[4]Afyonkarahisar Parkhayat Hospital, Ophthalmoloy Clinic, Afyonkarahisar, Turkey

Abstract

Background: The aim of this study was to evaluate the most common underlying genetic and clinical etiologies of retinitis pigmentosa (RP) disease in our geographical area.

Material and Method: In our archive, there are about 3000 patients who applied to our clinic between the years 2015-2021. The files of approximately 700 patients with a definitive genetic diagnosis were retrospectively scanned. A definitive genetic diagnosis was made in 22 of these patients. During our research, we collected some clinical parameters including the prenatal, natal, and postnatal history of the patients, history of surgery and seizures, and family history. In family history, we did a detailed pedigree with at least 3 generational analyses, questioned parental kinship, looked for similar members in families, and identified inheritance patterns of their disorder. We draw 3 generations pedigree and we collected peripheral venous blood samples from patients and sent them to a commercial lab for gene panels or WES. After obtaining the definitive genetic diagnosis of all patients, we compiled a table with the other parameters we questioned.

Results: As a result of our WES analysis in patients 1 and 2, homozygous c.1331_1332 dupAG/p. Thr445ArgfsTer10 Class 2 variant was detected in the POC1B gene of patient #2.In the RP panel 1 reports of patients 3 and 4, the genomic alteration of c.2254dupA (p.Ser752Lysfs*14) was detected in exon 15 of the ABCA4 (NM_000350) gene. Patient 5, EYS c.4964T>C heterozygous. Patient 6. SEMA4A C.1168A>G (heterozygous). Patient 7, SEMA4A C.1168A>G (heterozygous), RP1 c.5402C>T (heterozygous), CGNB1 c.1382C>T (heterozygous).Patient #8, . Heterozygous variation of p.Thr390Ala (c.1168A>G) in the SEMA4A gene is present.As a result of our WES analysis, a homozygous c.2021C>A/p.Pro674His Class 2 variant was detected in the RPGRIP1 gene of patient #9. Heterozygous c.119-2A>C Class 1 mutation was detected in the NR2E3 gene of patient 10. Homozygous c.271C>T/p.Gln91* Class 1 mutation was detected in the MFRP gene in patient 11. Patient #12 was diagnosed at the age of 7-8 years. When we look at the exome sequencing results, a homozygous mutation in the CNGB1 gene c.413-1G> of patient 13 was detected. Heterozygous p.Ser361Tyr (c.1082C>A) change detected in the ABCA4 gene of patient #14 was detected. The heterozygous p.Glu150Lys (c.448G>A) change detected in the RHO gene of patient #15 was pathogenic according to ClinVar database and in silico analysis. rated as. Prediagnosis was Bardet-Biedle Syndrome in patient 16. P.Gly244Asp change was detected in RPE65 gene of patients 17 and 18. Automated DNA sequencing of patient #19 and patient #20 results in a homozygous sequence variation in the coding sequence of the NR2E3 genes, a homozygous CGG>CAG nucleotide substitution, and an amino acid replacement of Arg311Gln. Heterozygous mutation was detected in the same gene region in patient 21 (fathers). Variation in NR2E3 is the most likely cause of these patients' eye condition, as it is a complete genotype and is strongly associated with RP in many published families. Genetic results on an allele of the BBS1 gene of patient 22 (chr11:66.278.121-66.291.364 (13.2kb)/ISCN: seq [GRCH37]11q13.2(66.278).121-66.291.364)x1). The other allele has a heterozygous point mutation (c.1424dupT p.Ser476fs-rs886039798).

Conclusıons: As determined in our study, the disease can be encountered with many different genetic etiologies. In this regard, patients undergoing genetic testing should be carefully examined for both SNP (single nucleotide polymorphism) and CNV (copy number variation).In addition, before genetic tests are performed, it should be well determined whether there is an isolated RP or an accompanying RP. In this respect, patients should be evaluated by making a detailed anamnesis and physical examination and drawing a pedigree containing at least 3 generations. Therefore, it was concluded that accompanying abnormalities should also be examined in the evaluation of retinitis pigmentosa anomalies.

Keywords: Retinitis pigmentosa, genetic mutations, genetic etiologies, gene therapies

Öz

Amaç: Bu çalışmanın amacı, coğrafi bölgemizdeki retinitis pigmentosa (RP) hastalığının en sık altta yatan genetik ve klinik etiyolojilerini değerlendirmektir.

Gereç ve Yöntem: Arşivimizde 2015-2021 yılları arasında kliniğimize başvuran yaklaşık 3000 hasta bulunmaktadır. Kesin genetik tanısı olan yaklaşık 700 hastanın dosyaları geriye dönük olarak tarandı. Bu hastaların 22'sine kesin genetik tanı konuldu. Araştırmamız sırasında hastaların doğum öncesi, doğum ve doğum sonrası öyküleri, ameliyat ve nöbet öyküsü ve aile öyküsü gibi bazı klinik parametreleri topladık. Aile öyküsünde, en az 3 kuşak analizi ile ayrıntılı bir soyağacı yaptık, ebeveyn akrabalığını sorguladık, ailelerde benzer üyeler aradık ve bozukluklarının kalıtım kalıplarını belirledik. 3 kuşak pedigri çizdik ve hastalardan periferik venöz kan örnekleri topladık ve bunları gen panelleri veya WES için ticari bir laboratuvara gönderdik. Tüm hastaların kesin genetik tanısını aldıktan sonra sorguladığımız diğer parametreleri içeren bir tablo oluşturduk.

Bulgular: 1 ve 2 numaralı hastalarda WES analizimiz sonucunda homozigot c.1331_1332 dupAG/p. Hasta #2'nin POC1B geninde Thr445ArgfsTer10 Sınıf 2 varyantı tespit edildi.3 ve 4 numaralı hastaların RP panel 1 raporlarında ABCA4 (NM_000350) geninin 15. ekzonunda c.2254dupA (p.Ser752Lysfs*14) genomik değişikliği tespit edildi. Hasta 5, EYS c.4964T>C heterozigot. Hasta 6,. SEMA4A C.1168A>G (heterozigot). Hasta 7, SEMA4A C.1168A>G (heterozigot), RP1 c.5402C>T (heterozigot), CGNB1 c.1382C>T (heterozigot). Hasta #8, . SEMA4A genindeki p.Thr390Ala'nın (c.1168A>G) heterozigot değişimi mevcut.WES analizimiz sonucunda hasta #9'un RPGRIP1 geninde homozigot c.2021C>A/p.Pro674His Sınıf 2 varyantı tespit edildi. 10 numaralı hastanın NR2E3 geninde heterozigot c.119-2A>C Sınıf 1 mutasyonu tespit edildi. 11 numaralı hastada MFRP geninde homozigot c.271C>T/p.Gln91* Sınıf 1 mutasyonu tespit edildi.Hasta #12, 7-8 yaşlarında teşhis edildi. Ekzom dizileme sonuçlarına baktığımızda 13 numaralı hastanın CNGB1 geni c.413-1G>bir homozigot mutasyon tespit edildi.Hasta #14'ün ABCA4 geninde saptanan heterozigot p.Ser361Tyr (c.1082C>A) değişikliği saptandı.15 numaralı hastanın RHO geninde saptanan heterozigot p.Glu150Lys (c.448G>A) değişikliği, ClinVar veri tabanına ve in silico analizine göre patojenik olarak puanlandı. 16 numaralı hastada Ön tanı Bardet-Biedle Sendromu olarak konuldu.17 ve 18 numaralı hastaların RPE65 geninde p.Gly244Asp değişikliği saptandı. Hasta #19 ve hasta #20'nin otomatik DNA dizilimi, NR2E3 genlerinin kodlama dizisinde bir homozigot dizi varyasyonu, bir homozigot CGG>CAG nükleotid ikamesi ve Arg311Gln'nin bir amino asit değişimi ile sonuçlanır. 21 numaralı hastada (babalar) aynı gen bölgesinde heterozigot mutasyon tespit edildi. NR2E3'teki varyasyon, tam bir genotip olduğundan ve birçok yayınlanmış ailede RP ile güçlü bir şekilde ilişkili olduğundan, bu hastaların göz durumunun en olası nedenidir.22 numaralı hastanın BBS1 geninin bir alelinde (chr11:66.278.121-66.291.364 (13.2kb)/ISCN: seq [GRCH37]11q13.2(66.278). 121-66.291.364)x1) genetik sonuçlarda. Diğer alel heterozigot nokta mutasyonuna sahiptir (c.1424dupT p.Ser476fs-rs886039798).

Sonuç: Çalışmamızda da belirlendiği üzere hastalık birçok farklı genetik etiyoloji ile karşımıza çıkabilmektedir. Bu bağlamda, genetik teste tabi tutulan hastalar hem SNP (tek nükleotid polimorfizmi) hem de CNV (kopya sayısı varyasyonu) açısından dikkatle incelenmelidir.Ayrıca genetik testler yapılmadan önce izole bir RP veya eşlik eden bir RP olup olmadığı iyi belirlenmelidir. Bu açıdan hastalar ayrıntılı bir anamnez ve fizik muayene yapılarak ve en az 3 kuşağı içeren soyağacı çizilerek değerlendirilmelidir. Bu nedenle retinitis pigmentosa anomalilerinin değerlendirilmesinde eşlik eden anormalliklerin de incelenmesi gerektiği sonucuna varıldı.

Anahtar Kelimeler: Retinitis pigmentosa, genetik mutasyonlar, genetik etiyolojiler, gen tedavileri

Corresponding *(İletişim)*: Özgür Eroğul, Afyonkarahisar Health Sciences University Faculty of Medicine, Department of Ophthalmology, Afyonkarahisar, Turkey
E-mail *(E-posta)*: ozgur_erogul@hotmail.com
Received *(Geliş Tarihi)*: 21.06.2022 **Accepted *(Kabul Tarihi)*:** 27.07.2022

INTRODUCTION

Retinitis pigmentosa (RP) is the most common hereditary retinal degeneration. It affects the respective rod and then cone photoreceptors. RP is manifested by poor rod photoreceptor function, night blindness, and a short peripheral visual field. Except for cystoid macular edema, it is seen in the latter case, as is the cone function seen in the central vision view. Classically described RP includes granular appearance due to atrophy of the fundus retinal pigment epithelium, bone speculation pigmentation, thinning of retinal vessels, and optic disc features. RP Mendelian can be seen in autosomal dominant, autosomal recessive or X-linked inheritance forms.

In autosomal recessive RP, non-destructive rhodopsin is encoded as a result of a null mutation in the rhodopsin gene or normal expression is blocked by regulatory mechanisms. Heterozygous individuals are clinically normal. In addition, β-phosphodiesterase gene mutations have also been identified as responsible.[1]

X-linked retinitis pigmentosa (XLRP) accounts for 5-20% of the cases of RP. Three disease-causing genes have been identified to date: retinitis pigmentosa GTPase regulator (RPGR; OMIM 312610), retinitis pigmentosa 2 (RP2; OMIM 312600). and the much rarer oral facial-digital syndrome type 1 (OFD1; OMIM 300170) gene.[2] Mutations within the RPGR gene, however, predominate and contribute to the highest rate of any RP locus identified to date. XLRP is particularly severe in males with early onset and rapid progression of vision loss, resulting in legal blindness by the end of the third decade. Female carriers do not usually report symptoms. However, it has long been appreciated that female carriers of XLRP can range from being asymptomatic to having a significant visual and retinal impairment. The carrier phenotype can vary accordingly with the ratio of X-inactivation.[3,4]

Autosomal Dominant Retinitis Pigmentosa (ADRP) is an inherited retinal degenerative disorder. It is characterized by progressive loss of photoreceptors, ultimately leading to irreversible loss of vision. This degeneration of photoreceptors begins in the peripheral retina, slowly progressing toward the central retina. In the cell context, rod photoreceptors are predominantly and primarily affected, leading to night blindness. In addition to this, eventually, there is degeneration of cones causing complete loss of vision. Degeneration of photoreceptors causes the relocation of retinal pigment epithelium to the inner retina. This process is clinically manifested as pigmented deposits in the peripheral retina on fundus examination.[5,6]

ADRP is caused by genetic mutations in the genes responsible for the basic functioning and maintenance of photoreceptors. Since the mutations are autosomal dominant, the disease phenotypes are observed even in the presence of a single mutated allele. These mutations can lead either to loss of function (LOF) or toxic gain of function (GOF) phenotypes. Irrespective of the nature of the mutation (deletion, missense, or non-sense) as well as the region of the gene in which these mutations occur (intronic or exonic), LOF mostly leads to a mutant protein which is usually unstable and gets degraded and the remaining wild-type protein is insufficient for proper functioning. Hence, a single vector-based gene supplementation approach might work for a spectrum of mutations in a given gene.[7,8]

Nonetheless, the effect of the GOF phenotype is mostly dependent on nature as well as the region of mutation in a given gene. The phenotypes vary from a mutant protein interfering with the function of a normal protein, gaining a new function by the mutant protein, or enhancing the degradation of the normal protein.[8]

It is deducted in mitochondrial digenic forms. But sporadic or simplex is the tightest form. The final method is to work bigger than gene selection. This optic is designed from an overview of the clinical, genetics, fundus photography, coherence tom, fundus autofluorescence, microperimetry, dark adaptometry, and ocular electrophysiological properties of RP. Night blindness in the early stage is often the main symptom. Firstly, mild night blindness is often overlooked by patients. There may be peripheral visual field defects in dim light at this stage. Especially if there is no family history (about half of the cases), it is difficult to diagnose during this period. Visual acuity is normal or below normal. Fundus examination is normal at baseline, retina arteriole attenuation is minimal and the optic disc is normal and the color vision is normal. The electroretinogram (ERG) is the key test. In most cases, scotopic shows reduced amplitude in the dominant b-wave under these conditions. With this at maximum ERG amplitude when the retina is partially affected ERG may appear normal with a decrease.[9]

Although the exact mechanisms that cause necrosis in patients with vision loss are not known in the pathophysiology of the disease, they reported that the finding that necrosis results in cone cell death brings one step closer to understanding this disease, and more importantly, it enables them to give new therapies to millions of people with growth factors and anti-apoptotic factors. When the related studies are evaluated, some growth factors such as ciliary neurotrophic factor (CNTF), glial-derived neurotrophic factor (GDNF), cardiotropin-1, brain-derived neurotrophic factor (BDNF), and basic fibroblast growth factor (bFGF) have been tried in the treatment of RP in some animal models. However, besides the side effects of these factors such as retinal neovascularization and cataracts.[10]

It has been determined that they cause a decrease in the ERG response of the retina by an unknown toxic mechanism. In addition, in some animal models, bcl-2 gene transfer from anti-apoptotic factors and the use of caspase inhibitor peptides have been shown to slow down photoreceptor cell death. Death caspases activate cytoplasmic endonucleases and proteases, thereby reducing nuclear and cytoskeletal proteins. New studies on caspase-3, caspase-6 and caspase-7 are ongoing. By using microphotodiode arrays that replace photoreceptors, clinical studies on retinal prostheses that stimulate the retina, optic nerve or visual cortex are one of the most popular studies today. In addition, in animal models, retinal cells, photoreceptor

layers, RPE grafts, or tissue of the entire retina transplantation and retinal or other. Studies on embryonic or adult stem cells from tissues continue.[11]

Currently, genetic technologies have been rapidly growing and the association between human genetic variation and disease has been reconsidered. Hereditary retinal diseases constitute a large proportion of retinal pathologies. Increasing knowledge about inheritance patterns and mutations, as well as the rapidly growing novel information as a result of the utilization of new genetic technologies lead to the definition of novel clinical entities together with options for the diagnosis and treatment. This review focuses on inheritance patterns of hereditary retinal diseases and mutations with recent technological improvements.[12]

MATERIAL AND METHOD

In our archive, there are about 3000 patients who applied to our clinic between the years 2015-2021. The study was conducted in line with the principles of the Declaration of Helsinki, and the method and purpose of the study were explained to all participants in detail, and informed consent was obtained from each patient. The study was carried out with the permission of Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (Date: 03.12.2021, Decision No: 2021/13). The files of approximately 700 patients with a definitive genetic diagnosis were retrospectively scanned. A patient with an RP anomaly has been identified. A definitive genetic diagnosis was made in 22 of these patients. In this study, this patient was evaluated and presented to the literature. The inclusion criteria for this study were to have RP anomalies. Patients diagnosed with a disease other than retinitis pigmentosa that may affect the retina were excluded from the study. During our research, we collected some clinical parameters including the prenatal, natal, and postnatal history of the patients, history of surgery and seizures, and family history. In family history, we did a detailed pedigree with at least 3 generational analyses, questioned parental kinship, looked for similar members in families, and identified inheritance patterns of their disorder.

We draw 3 generations pedigree and we collected peripheral venous blood samples from patients and sent them to a commercial lab for gene panels or WES. After obtaining the definitive genetic diagnosis of all patients, we compiled a table with the other parameters we questioned.

RESULTS

The genetic etiologies of 22 patients with definite genetic etiology are given in **Table 1**. Clinical findings of 22 patients are given in **Table 2**.

Patients #1 and #2 go to the park to play ball during the day, but they cannot play ball. They can hardly see beyond 2 meters. Also, patient #2 is mixing colors. He has difficulty learning. There is parental consanguinity. As a result of our WES analysis, homozygous c.1331_1332 dupAG/p. Thr445ArgfsTer10 Class 2 variant was detected in the POC1B gene of patient #2.

Patients #3 and #4 were admitted with the suspicion of RP. When the results of the next-generation DNA sequencing were examined, in the RP panel 1 reports of both patients, the genomic change of c.2254dupA (p.Ser752Lysfs*14) in the 15th exon of the ABCA4 (NM_000350) gene was found to be homozygous. This genomic alteration was evaluated as "Likely Pathogenic" according to the ACMG-2015* criteria. This result is consistent with the clinical findings in patients.

Patient #5 has been experiencing visual loss since birth. He can finally see 10% bilaterally. He had RP from birth. EYS c.4964T>C heterozygous. Patient #6 (mother) has an RP clinic. It looks lighter. It started after the age of 20. SEMA4A C.1168A>G (heterozygous). Patient #7 (uncle) has had RP since birth. The clinic was available. SEMA4A C.1168A>G (heterozygous), RP1 c.5402C>T (heterozygous), CGNB1 c.1382C>T (heterozygous).

Patient #8 has had vision problems for 13 years. He was diagnosed with RP 13 years ago. His uncle also has RP. Although the heterozygous change of p.Thr390Ala (c.1168A>G) in the SEMA4A gene is classified as benign according to ACMG criteria, it was evaluated as VUS (Variant Uncertain Significance) because there were conflicting data in in-silico analyzes and there was no data regarding its clinical significance in the ClinVar database.

Patient #9 has congenital RP. After the age of 23, his vision decreased to 6%. Patient #10 has RP. She was seeing 5% from birth. As a result of our WES analysis, a homozygous c.2021C>A / p.Pro674His Class 2 variant was detected in the RPGRIP1 gene of patient #9. Heterozygous c.119-2A>C Class 1 mutation was detected in the NR2E3 gene of patient #10.

Patient #11 was admitted to our hospital 2 years ago with the complaint of narrowing of the visual field in both eyes. The patient was diagnosed with RP. As a result of our WES analysis, a homozygous c.271C>T / p.Gln91* Class 1 mutation was detected in the patient's MFRP gene. It is expected that this result will lead to microphthalmia, and isolated 5 clinics in the patient.

Patient #12 was diagnosed around the age of 7-8 years. Patient #13 (father) was diagnosed at a similar age. When we look at the exome sequencing results, patient #13's CNGB1 gene c.413-1G>A homozygous mutation was detected.

It was noticed that patient #14 could not see the blackboard in the 2nd grade of primary school. It currently has a 60% vision rate. Patient #14 was diagnosed with vision loss when she went to primary school with a headache in her second grade. When we look at the results of the sequence analysis, the heterozygous p.Ser361Tyr (c.1082C>A) change detected in the ABCA4 gene of Patient #14 is scored as VUS (Variant Uncertain Significance) according to the ACMG criteria and in the ClinVar database. The heterozygous p.Glu150Lys (c.448G>A) change detected in the RHO gene of patient #15 was scored as pathogenic according to the ClinVar database and in silico analysis. It is classified as a possible pathogenic change

according to the ACMG criteria. In addition, the heterozygous p.Ser361Tyr (c.1082C>A) change detected in the ABCA4 gene is scored as VUS (Variant Uncertain Significance) according to the ClinVar database and ACMG criteria.

Patient #16 has mental retardation, bilateral polydactyly of the feet, RP, hyporeflexia, and growth retardation. He can't walk or talk. He also has microretrognathia, left side outward strabismus, and dysmetria dysdiodykinesia. The preliminary diagnosis was made as Bardet-Biedle Syndrome.

Patient #17 has congenital vision loss. Vision decreased over time. Currently 10% sight is available. Patient #18 (sibling) has less vision. There is parental consanguinity. When we look at the results of the sequence analysis, the homozygous p.Gly244Asp change detected in the RPE65 gene of patient #17 has not been defined before and there is no data regarding its clinical significance in the literature. However, it is classified as potentially pathogenic according to in-silico evaluations and ACMG criteria. The homozygous p.Gly244Asp change detected in the RPE65 gene in patient #18 has not been described before, and there is no data regarding its clinical significance in the literature. However, it is classified as potentially pathogenic according to in-silico evaluations and ACMG criteria.

Table 1. Genetic Analysis Results and Heredity

Case ID	Gene(s)	OMIM	Mode of inheritance	Consanguineous marriage	Is there another affected individual?	Mutation(s)	Zygosity	Genetic diagnosis
1				1st degree cousin marriage	old-case2) vision problem, inability to			Retinitis pigmentosa
2	POC1B	615973	autosomal-recessive	1st degree cousin marriage	old-case1) vision problem	p.Thr445ArgfsTer10/ Class2	Homozygous	Cone-rod distrofi, Retinitis pigmentosa
3	ABCA4			.	Sister (16 years old-case4)	c.2254dupA(pSer752 1Lysfs*14)	Homozygous	Retinitis pigmentosa
4	ABCA4				Brother (17 years old-case3)	c.2254dupA(pSer752 1Lysfs*14)	Homozygous	Retinitis pigmentosa
5	EYS			Same village	Mother (case6) and uncle (case7)	c.4964T>C	Heterozygotes	Retinitis pigmentosa
6	SEMA4A			sAme village	Son (case5) and brother (case7)	C.1168A>G	Heterozygotes	Retinitis pigmentosa
7	SEMA4A, RP1, CGNB1			Same village	Sister (case6) and Nephew (case5)	C.1168A>G, c.5402C>T, c.1382C>T	Heterozygotes	Retinitis pigmentosa
8	SEMA4A			1St degree cousin marriage	Uncle (RP)	p.Thr390Ala (c.1168A>G)	Heterozygotes	Retinitis pigmentosa
9	RPGRIP1	613826	autosomal-recessive	Same village	No	c.2021C>A/p.Pro674His /Class2	Homozygous	Retinitis pigmentosa
10	NR2E3	611131	autosomal-dominant	1st degree cousin marriage	No	c.119-2A>C/ Class	Heterozygotes	Retinitis pigmentosa
11	MFRP	611040	autosomal recessive	1St degree cousin marriage	Grandfather-vision problem	c.271C>T / p.Gln91* Class1	Homozygous	Retinitis pigmentosa
12	CNGB1			1St degree cousin marriage	Father (case13)	c.413-1G>A	Homozygous	Retinitis pigmentosa
13	CNGB1				Son (case12)	c.413-1G>A(p.(Cys139fs))	Homozygous	Retinitis pigmentosa
14	ABCA4			1st degree cousin mar	case15) vision problem	p.Ser361Tyr (c.1082C>A)	Heterozygotes	Retinitis pigmentosa
15	ABCA4, RHO			1st degree cousin mar	old-case14) %60 vision rate	(c.1082C>A),p.Glu150Lys	Heterozygotes	Retinitis pigmentosa
16			autosomal recessive	2nd degree cousin ma	Aunt (Dead) inability to walk and talk			bilateral polydactyly, hyporeflexia
17	RPE65			1st degree cousin mar	1 female sibling (case18)	p.Gly244Asp (c.731G>A)	Homozygous	Retinitis pigmentosa
18	RPE65			1st degree cousin mar	1 female sibling (case17)	p.Gly244Asp (c.731G>A)	Homozygous	Retinitis pigmentosa
19	NR2E3				(case20) father (case21)	Arg311Gln CGG>CAG	Homozygous	Retinitis pigmentosa
20	NR2E3				(case19) father (case21)	Arg311Gln CGG>CAG	Homozygous	Retinitis pigmentosa
21	NR2E3				Two son (case19-case20)	Arg311Gln CGG>CAG	Heterozygotes	Retinitis pigmentosa
22	BBS1	209900				c.1424dupT p.Ser476fs rs886039798 mutation / chr11:66.278.121-66.291.364 delesion	Heterozygotes	Retinitis pigmentosa

Table 2. Patient Clinical Informations and Findings

Case ID	Complaints	Birth	Seizure	Operation(s)	Findings
1	Retinitis pigmentosa	2005	no	Strabismus	He can't see when he goes to the park to play ball in the daytime. He can't play ball in the park. In the daytime, he cannot see beyond 2
2	Retinitis pigmentosa	2007	no	no	In the daytime, he can hardly see beyond 2 meters in the sun. He can't see far at night. He also mixes colors and has difficulty
3	Retinitis pigmentosa	2005			
4	Retinitis pigmentosa	2006			
5	Retinitis pigmentosa	1991	no	no	He had vision loss from birth. Last can see 10% bilaterally
6	Retinitis pigmentosa				It is milder. His complaints started after the age of 20.
7	Retinitis pigmentosa				He has had retinitis pigmentosa since birth
8	Retinitis pigmentosa	1986	no	no	He has been suffering from vision problems for 13 years. The patient was diagnosed with retinitis pigmentosa 13 years ago.
9	Retinitis pigmentosa	1990	no	no	He had congenital retinitis pigmentosa. After the age of 23, the vision rate decreases to 6%.
10	Retinitis pigmentosa	1995	no	Cataract	She sees 5% at birth
11	Retinitis pigmentosa	1994	no	no	Two years ago, she applied with the complaint of narrowing of the visual field in both eyes, and was diagnosed with retinitis
12	Retinitis pigmentosa	1993			The disease was noticed around the age of 7-8 years.
13	Retinitis pigmentosa	1968			It was noticed around the age of 7- 8, similar to his son.
14	Retinitis pigmentosa	1995	no	no	In elementary school, her teacher noticed that she couldn't see the blackboard. Currently seeing 60%.
15	Retinitis pigmentosa	2002	no	no	When she went to the doctor with a headache complaint in primary school, she was told that she had a vision problem.
16	Retinitis pigmentosa	1997	no	no	There are polydactyly, pes planus, microretrognathia, left eye outward squint, and dysmetria- dysdiodykinesia in the feet.
17	Retinitis pigmentosa	1991	no	no	There is congenital vision loss. Over time, her vision decreased even more. She used to go to school on her own, but now she
18	Retinitis pigmentosa	1983	no	no	She sees less than her sister (case17).
19	Retinitis pigmentosa	1967			
20	Retinitis pigmentosa	1967			
21	Retinitis pigmentosa	1934			
22	Retinitis pigmentosa	1995			Bardet-Biedl syndrome, Obesity, Retinitis pigmentosa, Polydactyly, Motor regression

Automated DNA sequencing of patient #19 and patient #20 results in a homozygous sequence variation in the coding sequence of the NR2E3 genes, a homozygous CGG>CAG nucleotide substitution, and an amino acid change of Arg311Gln. It revealed 1 possible high-penetration disease-causing sequence variation in the NR2E3 gene and 1 possible disease-causing sequence variation in each of the CDHR1, IFT140 and MERTK genes. Heterozygous mutation in the same gene region was detected in patient #21 (fathers). Variation in NR2E3 is the most likely cause of these patients' eye condition, as it is a complete genotype and has been strongly associated with RP in many published families.

When we look at the clinical examination of patient #22, there is obesity, polydactyly, motor regression and RP. These results show us that the patient is compatible with Bardet-Biedle syndrome. In addition, when we examined the patient for RP, there was a heterozygous deletion in an allele of the BBS1 gene (chr11:66.278.121-66.291.364 (13.2kb)/ ISCN: seq [GRCH37]11q13.2(66.278.121-66.291.364) x1) in the genetic results. while the other allele has a heterozygous point mutation (c.1424dupT-p.Ser476fs-rs886039798).

DISCUSSION

In this study, the purpose was to investigate patients with RP and to find the most common underlying genetic and clinical etiologies in our geographic area. In this process, the phenotypes and accompanying abnormalities helped us a lot during our diagnosis period and to choose the most proper testing, such as specific single-gene sequencing, panel testing, or WES. Therefore, it was concluded that it is essential to assess the accompanying abnormalities in the evaluation of retinitis pigmentosa anomalies because they can be isolated or as a part of a syndrome and can lead us to a specific syndrome or not.

It is very important to determine the inheritance pattern in RP disease. Because as a result of the genetic test we do, you try to determine a dystrophy type according to that heredity. For this reason, we drew pedigrees containing at least 3 generations for all our patients. When we look at the genetic analysis results of our patients, we see that 13 of our patients have homozygous changes and 9 patients have heterozygous changes. Although clinical symptoms of RP were present in one of our patients, Bardet-Biedle syndrome was diagnosed as a preliminary diagnosis in the patient.

In addition, we questioned whether there was another retinitis RP in the family in the pedigree analysis that included 3 generations. Because the presence of more than one person in the family suggests dominant inheritance, while its presence only in males suggests X-linked recessive inheritance. Recessive inheritance is suggested if there is a horizontal inheritance or if the individuals affected are few and if there is consanguinity between the parents. In this respect, we questioned the existence of another affected individual in the family. When we look at the patient data we used in the study, we see that patient #5's mother (patient #6) and uncle (patient #7) had the same disease. It was also found that the distant relative of his mother and uncle (patient #8) had the same disease. Patient #11's grandfather has the same disease. In patient #12's father (patient #13) and uncle; patient #19's father (patient #21) and sibling (patient #20) were diagnosed with RP. When we look at the other patients for whom we have data, it has been reported by patients who do not have a family history of RP. Our study shows that this disease can also occur in different members of the family. Similar to our study, Dr. Al-Byoud et al. studied 5 related Jordanian families in their study. In their results, they reported that this disease showed an autosomal recessive inheritance pattern and was diagnosed in every affected member of the family.[13]

Previous surgical operations are also important when researching the clinical data of patients. Because it gives clues in terms of chronic diseases they have. In this respect, we questioned the surgical procedures and chronic diseases of our patients. When we look at the surgery information of the patients, it is known that patient #1 had strabismus surgery and tonsillectomy and patient #10 had cataract surgery in her left eye. We have information that other patients do not have a history of surgery. In our study, when we questioned the operation status of our patients, we saw that two of our patients had surgery. Dr. Chatterjee et al., in their study, reported that RP patients had an increase in their visual acuity after surgery.[14] In our study, when we questioned the operation status of our patients, we saw that two of our patients had cataract surgery. A cataract is an important secondary cause of visual impairment in RP. It is characterized by early onset and the most common morphological type reported in the literature is posterior subcapsular cataract.[15-19] Along with the onset of cataracts, the most frequently affected visual function in patients with RP is contrast sensitivity, cataract progression, and a general decrease in vision. Most patients with RP are young to middle-aged adults. Therefore, the onset of cataracts leads to worsening of vision in these patients.[20] Dr. Chatterjee et al., in their study, reported that retinitis pigmentosa patients had an increase in their visual acuity after cataract surgery.[14] In our study, however, there is not enough data on whether the rate of vision increases after surgery.

The age of onset of RP varies according to the affected individual. When we consider the age of onset of the patients, it was reported that patient #2 did not have a definite age of onset, but according to his mother's words, he saw normal at home when he was 5 years old, but only saw his own area outside. It is known that patient #5, patient #7 and patient #17 have a congenital visual loss. It started after the age of 20 in patient #6, patient #8 had vision problems for 16 years and was diagnosed 16 years ago, patient #9 had congenital vision loss and the rate of vision decreased to 6% after 23 years of age. It is stated in our data that #10 has 5% vision since birth, that this disease was diagnosed when he was 24 years old in patient #11, and it appeared in patient #12 and his father (patient #13) at the age of 7-8 years. In the remaining patients, the age of onset of this disease is unknown. In our study, we examined the age of onset of the disease. When we review the literature, we see that the age of onset is not emphasized in the articles we have reviewed.

Gene therapies for the affected gene have started in RP disease. In this respect, it is of great importance to diagnose the defective gene by performing genetic testing. Now, we examine, respectively, some of the mutations we have detected and the comparison of the effects of these mutations in the literature. The most common gene mutation in our study was the NR2E3 gene mutation. It appeared in the father and his two sons. It also occurred in another patient completely independent of the family. While it was in the form of a heterozygous mutation in the father and the other patient, it was homozygous in two children. Similar to our study, Dr. Blanco-Kelly et al. studied 201 patients with ADRP in their study. These patients were completely independent of each other and in their results, they found that 24 patients had NR2E3 gene mutations.

They noted that this situation led to a prevalence of 3.5%.[21] After this gene, the most common mutation is SEMA4A heterozygous gene mutation. The difference here is that while this mutation is observed in the mother, uncle, and distant relative, the gene mutation occurring in the patient is EYS heterozygous gene mutation. Examining the SEMA4 gene mutation outside of our study, Dr. Abid and colleagues found that this gene causes not only RP but also cone-rod dystrophy. In addition, they also revealed in their study that this gene mutation occurred in the conserved semaphorin area, unlike us.[22] Subsequent mutations are ABCA4 homozygous gene mutation, CNGB1 homozygous gene mutation, and RPE65 homozygous gene mutation. These mutations are seen among family members as a result of kinship ties.

In recent years, gene therapy-based drugs have been offered to patients with RP, especially those with RPE65 homozygous mutations. This is a turning point for the use of gene therapy in RP disease. We detected p.Gly244Asp (c.731G>A) homozygous mutation in the RPE65 gene of patient#17 and patient#18 from our patients. Consistent with recessive inheritance, patients have first-degree cousin marriages in their parents. Both patients were diagnosed at an early age. Dr. Sun et al. in their study, evaluated a total of 116 patients, including 105 unrelated patients, for ABCA4 gene mutation. In this study, they also examined different variants of the ABCA4 gene mutation in patients, unlike us. As a result, they identified 129 different pathogenic ABCA4 variants.[23] Dr. Issa et al. examined 9 patients for CNGB1 gene mutation in their study. In their results, they revealed 5 new mutations in the CNGB1 gene and 5 mutations previously revealed in other studies.[24] Dr. Jauregui et al. investigated the RPE65 gene mutation in the ADRP disease in their study. They included a 67-year-old male patient in their study. They followed the patient for 2 years. At the end of the 2-year study, they reported that the rate of progression of the disease was slow and mild.[25]

In our study, we detected isolated RP patients, as well as syndromic RP patients. In Patient#22, we detected a mutation in the BBS1 gene in one allele and a deletion in the other allele containing the BBS1 gene. Since the patient's clinic was compatible with Bardet-Biedle syndrome, it was a good case for us in terms of diagnosis. Bardet-Biedle syndrome is one of the autosomal recessive inherited genetic obesity syndromes, which is characterized by cardinal findings of retinal dystrophy, polydactyly, obesity, hypogonadism, and kidney anomalies, which is considered among the "ciliopathy" pathologies today. Our patient was also clinically compatible with BBS. Interestingly, we detected recessive BBS1 syndrome. Because deletion (heterozygous chr11:66.278.121-66.291.364 (13.2kb) ISCN: seq [GRCH37] 11Q13.2(66.278.121-66.291.364)X1) in one allele of our patient whose parents were unrelated, point mutation (The BBS1 gene (exon1-11)/c.1424dupT/pSer476fsrs886039798-heterozygous) was our detection.

CONCLUSION

As determined in our study, the disease can be encountered with many different genetic etiologies. In this regard, patients undergoing genetic testing should be carefully examined for both SNP (single nucleotide polymorphism) and CNV (copy number variation).

In addition, whether there is isolated RP or an RP accompanying the syndrome should be well-identified before genetic testing is performed. In this respect, patients should be evaluated by applying a detailed anamnesis and physical examination, and drawing a pedigree that includes at least 3 generations

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (Date: 03.12.2021, Decision No: 2021/13).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. Rosenfeld PJ, Cowley GS, McGee TL, Sandberg MA, Berson EL, Dryja TP. A null mutation in the rhodopsin gene causes rod photoreceptor dysfunction and autosomal recessive retinitis pigmentosa. Nat Genet 1992;1(3):209-13.
2. Ott J, Bhattacharya S, Chen JD, et al. Localizing multiple X chromosome-linked retinitis pigmentosa loci using multilocus homogeneity tests. Proc Natl Acad Sci 1990;87(2):701-4.
3. Flaxel CJ, Jay M, Thiselton DL, et al. Difference between RP2 and RP3 phenotypes in X linked retinitis pigmentosa. Br J Ophthalmol 1999;83(10):1144-8.
4. Churchill JD, Bowne SJ, Sullivan LS, et al. Mutations in the X-linked retinitis pigmentosa genes RPGR and RP2 found in 8.5% of families with a provisional diagnosis of autosomal dominant retinitis pigmentosa. Invest Ophthalmol Vis Sci 2013;54(2):1411-6.
5. Narasimhan I, Murali A, Subramanian K, Ramalingam S, Parameswaran S. Autosomal dominant retinitis pigmentosa with toxic gain of function: Mechanisms and therapeutics. Eur J Ophthalmol 2021;31(2):304-20.
6. Jaissle GB, May CA, van de Pavert SA, et al. Bone spicule pigment formation in retinitis pigmentosa: insights from a mouse model. Graefe's Arch Clin Exp Ophthalmol 2010;248(8):1063-70.
7. Tsang SH, Sharma T. Autosomal dominant retinitis pigmentosa. Atlas Inherit Retin Dis. Published online 2018:69-77.
8. Daiger SP, Bowne SJ, Sullivan LS. Genes and mutations causing autosomal dominant retinitis pigmentosa. Cold Spring Harb Perspect Med 2015;5(10):a017129.

9. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet 2006;368(9549):1795-809.
10. O'Neal TB, Luther EE. Retinitis pigmentosa. In: StatPearls [Internet]. StatPearls Publishing; 2021.
11. Wang AL, Knight DK, Thanh-thao TV, Mehta MC. Retinitis pigmentosa: review of current treatment. Int Ophthalmol Clin 2019;59(1):263-80.
12. Sharon D, Ben-Yosef T, Goldenberg-Cohen N, et al. A nationwide genetic analysis of inherited retinal diseases in Israel as assessed by the Israeli inherited retinal disease consortium (IIRDC). Hum Mutat 2020;41(1):140-9.
13. Al-Bdour M, Pauleck S, Dardas Z, et al. Clinical heterogeneity in retinitis pigmentosa caused by variants in RP1 and RLBP1 in five extended consanguineous pedigrees. Mol Vis 2020;26:445.
14. Chatterjee S, Agrawal D, Agrawal D, Parchand SM, Sahu A. Cataract surgery in retinitis pigmentosa. Indian J Ophthalmol 2021;69(7):1753.
15. Knapp A. Cataract in retinitis pigmentosa; its pathology and treatment. Trans Am Ophthalmol Soc 1918;16:59.
16. Heckenlively J. The frequency of posterior subcapsular cataract in the hereditary retinal degenerations. Am J Ophthalmol 1982;93(6):733-8.
17. Pruett RC. Retinitis pigmentosa: clinical observations and correlations. Trans Am Ophthalmol Soc 1983;81:693.
18. Fishman GA, Anderson RJ, Lourenco P. Prevalence of posterior subcapsular lens opacities in patients with retinitis pigmentosa. Br J Ophthalmol 1985;69(4):263-6.
19. Liew G, Strong S, Bradley P, et al. Prevalence of cystoid macular oedema, epiretinal membrane and cataract in retinitis pigmentosa. Br J Ophthalmol 2019;103(8):1163-6.
20. Oomachi K, Ogata K, Sugawara T, Hagiwara A, Hata A, Yamamoto S. Evaluation of contrast visual acuity in patients with retinitis pigmentosa. Clin Ophthalmol (Auckland, NZ) 2011;5:1459.
21. Blanco-Kelly F, García Hoyos M, Lopez Martinez MA, et al. Dominant retinitis pigmentosa, p. Gly56Arg mutation in NR2E3: Phenotype in a large cohort of 24 cases. PLoS One 2016;11(2):e0149473.
22. Abid A, Ismail M, Mehdi SQ, Khaliq S. Identification of novel mutations in the SEMA4A gene associated with retinal degenerative diseases. J Med Genet 2006;43(4):378-81.
23. Sun Z, Yang L, Li H, et al. Clinical and genetic analysis of the ABCA4 gene associated retinal dystrophy in a large Chinese cohort. Exp Eye Res 2021;202:108389.
24. Issa PC, Reuter P, Kühlewein L, et al. Olfactory dysfunction in patients with CNGB1-associated retinitis pigmentosa. Jama Ophthalmol 2018;136(7):761-9.
25. Jauregui R, Park KS, Tsang SH. Two-year progression analysis of RPE65 autosomal dominant retinitis pigmentosa. Ophthalmic Genet 2018;39(4):544-9.