의학석사 학위논문

한국인 소아 유전성 망막질환 환자의 유전 분석

2021년 8월

서울대학교 대학원

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배 기 웅

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이 논문을 의학석사 학위논문으로 제출함

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배기웅의 의학석사 학위논문을 인준함

2021년 7월

위 원 장 구 승 엽

부위원장 유 형 곤

위 원 권 성 근

Abstract

Genetic Analysis of Korean Pediatric Patients with Inherited Retinal Disease

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Inherited retinal disease (IRD) is a group of predominantly monogenic disorders which have clinically and genetically heterogeneous origins. IRD can cause severe visual deterioration or blindness and is one of the leading cause of blindness worldwide. Timely and precise diagnosis is important for patient prognosis and counseling.

The eye is an ideal target for regenerative medicine. Since the eyeball is directly exposed to the outside, it is relatively easy to approach and evaluate for therapeutic intervention and treatment effect. Also, it has a compact size and is known as the immune-privileged space, which means there is lower risk of graft rejection in the eyeball. Therefore, a small dose of

cells and/or therapeutic agents are sufficient.

Recently, voretigene neparvovec-rzyl (LUXTURNA®) was approved by the Food and Drug Administration as the first gene therapy for the treatment of patients with biallelic RPE65 mutation-associated retinal dystrophy. Also, there is a report that retinal pigment epithelium (RPE) cells could be differentiated from induced pluripotent stem cells (iPSCs), originating from the skin fibroblasts of patients with macular degeneration. The authors successfully implanted the autologous iPSC-derived RPE cells sheet into the eye and no adverse event was reported after 1 year of follow-up. The result of this study shows the safety of stem cell transplantation into the diseased human eye. However, the clinical efficacy has not yet been proven, and several preliminary studies investigating RPE disease are ongoing.

Both gene therapy and stem cell treatment share the therapeutic goal to restore the diseased genes or cells. Moreover, IRD is caused by genetic abnormalities in the retinal structure and/or function. Therefore, an understanding of the genetic background of IRD may be helpful in the investigation of stem cell treatment. Here, we studied the genetic etiology and phenotypes of IRD in Korean children.

Pediatric patients with IRD (n = 121) who visited Seoul National University Hospital between 2011 and 2020 were investigated. Subjects underwent genetic screening,

including targeted gene sequencing, next-generation sequencing-based gene panel, or whole exome sequencing, to investigate the causative mutations. A total of 121 probands (86 men and 35 women) with 116 families were involved.

The median age at which the patients developed visual symptoms related to IRD was 6.9 ± 5.6 years (range, 0–18 years). Retinitis pigmentosa (43.0%), X-linked retinoschisis (28.1%), and Stargardt disease (5.8%) were the common clinical diagnoses in this cohort. Among all the subjects, the genetic etiology of diseases was confirmed in 67 (55.4%) patients and pathogenic mutations were identified in 23 retinal genes.

The results of this study will serve as a basis for genetic counseling of IRD patients and their family members and lay the cornerstone for the progress of the regenerative medicine in the future.

Keywords : inherited retinal disease, retinitis pigmentosa, gene panel, next-generation sequencing, whole exome sequencing

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Chapter 1. Introduction

Inherited retinal disease (IRD) is a group of disorders which have clinically and genetically heterogeneous origins including retinitis pigmentosa (RP) and other macular dystrophies. These conditions are related to genetic mutations that cause loss or dysfunction of the photoreceptor cells, retinal pigment epithelial cells, or both [1-3].

The retina is the innermost region of the eyeball that comprises multi-layers of neural cells. The retina converts the light into electrical signals, which are transferred to the visual cortex of the brain through the optic nerve to create visual perception. The retina therefore plays a key role in vision formation. The photoreceptor cells consist of rods and cones, which are located in the outer layer of the retina. They are specialized cells for light perception and photo-transduction. The retinal pigment epithelium (RPE) is placed outside the photoreceptor layer and provides functional and metabolic support to the rods and cones.

Patients with IRD have pigmentary abnormalities in their retina as shown on fundus examination, as well as loss and thinning of the outer retinal layers as observed on optical coherence tomography scans. Several common visual symptoms are associated with IRD, including night blindness and loss of peripheral visual field, while central vision can also

decrease as the disease progresses (Figure 1).

The eye is an ideal target for regenerative medicine. Since the eyeball is directly exposed to the outside, it is relatively easy to approach and evaluate for therapeutic intervention and treatment effect. Also, it has a compact size and is known as the immune-privileged space, which means there is lower risk of graft rejection in the eyeball. Therefore, a small dose of cells and/or therapeutic agents are sufficient.

To date, more than 270 genes linked with IRD have been identified in the Retinal Information Network (https://sph.uth.edu/Retnet/). Considering an estimated incidence of up to 1:2000, IRD is a major cause of visual impairment in children [4,5]. Timely recognition and appropriate management of IRD can have significant implications on the health and development of children [6]. Currently, advances in sequencing technologies, especially next-generation sequencing methods, have accelerated the diagnosis and have facilitated a precise medical approach to genetic diseases [7,8].

As the major advances in diagnosis and understanding for the pathologic basis of IRD, the biotechnologies including the gene transfer and the cell transplantation have also evolved and created the condition that previously blinding retinal diseases may be curable. Stem cell treatment for retinal degeneration shows therapeutic effects after delivery into the precise

location in the eye; it shows its ability to improve the micro-environment and regenerate, reverse, or neuro-protect against the disease processes [9].

Mandai et al. [10] reported that RPE cells could be differentiated from induced pluripotent stem cells (iPSCs), originating from the skin fibroblasts of patients with macular degeneration. They implanted the autologous iPSC-derived RPE cells sheet into the eye of a patient via subretinal approach. The vision did not improve or deteriorate. The transplanted RPE sheet remained intact, and no adverse event was reported after 1 year of follow-up. The result of this study shows the safety of stem cell transplantation into the diseased human eye. However, the clinical efficacy has not yet been proven, and several preliminary studies investigating RPE disease are ongoing [ClinicalTrials.gov number, NCT-04604899, 02464436].

Recently, voretigene neparvovec-rzyl (LUXTURNA®, Spark Therapeutics, Inc., Philadelphia, PA, USA) was approved by the Food and Drug Administration as the first gene therapy for the treatment of patients with biallelic RPE65 mutation-associated retinal dystrophy [11]. The result of this study shows a definite safety profile and significant visual improvements in the subjects. For the upcoming growth in gene therapy, identification of causative mutations in IRD is necessary for accurate diagnosis and should be preceded by the

use of new treatment options.

Stem cell transplantation for IRD is being actively researched and has a promising future. However, the research is now in its early stages, and the efficacy is still unclear, as opposed to that of gene therapy. Gene therapy and stem cell treatment are overlapping in that both share the therapeutic goal to restore the diseased genes or cells. Therefore, an understanding of gene therapy and the genetic background for IRD may be meaningful in the investigation of stem cell treatment.

The aim of this study was to assess the genetic etiology and phenotype of childhood-onset of IRD in Korea.

Chapter 2. Materials and Methods

2.1. Patients

This study was approved by the Institutional Review Board of the Seoul National University Hospital (SNUH) and adhered to the tenets of the Declaration of Helsinki (IRB number H-1107-101-370, approval date: January 19, 2021). Medical records of the patients referred to the SNUH clinic for IRD between 2011 and 2020 were reviewed, and probands that met the following inclusion criteria were selected: patients under 30 years of age with visual symptoms related to IRD, including night blindness, decreased vision, or strabismus, which had developed before the age of 18 years were enrolled in this study.

A full clinical history was recorded, and a complete ophthalmic examination was performed by retinal specialists on all probands and their available family members. The ophthalmic examination included visual acuity assessment, intraocular pressure measurement, and slit lamp examination of the anterior and posterior compartments. All the patients also underwent color fundus photography, spectral domain optical coherence tomography, and full-field electroretinography.

2.2. Genetic tests

Molecular genetic tests were performed using blood samples obtained from all patients and their family members, including their parents and siblings, in available cases. The genetic testing included direct targeted gene sequencing, next-generation sequencing (NGS)-based gene panel, and whole exome sequencing (WES). Direct gene sequencing of the RS1 was performed in patients who were clinically diagnosed with juvenile retinoschisis. NGS-based gene panel analysis and WES were conducted in subjects with RP, allied retinal degeneration, or macular dystrophies. The causative variants were verified based on the clinical features and inheritance patterns after genetic analysis.

2.2.1. RS1 gene sequencing

RS1 gene sequencing was performed as previously reported [12-14]. Genomic DNA was extracted from the peripheral blood of the patients, and polymerase chain reaction (PCR) followed by Sanger sequencing was performed for all six coding exons of RS1. Oligonucleotide primers for the flanking intron/untranslated region (UTR) sequences were designed and PCR was run using these primers. To identify the sequence variations, a wild type reference sequence of RS1 (NM_000330.2) was used; the variations were numbered

based on the cDNA sequence, with +1 corresponding to the first nucleotide of the initiation codon (ATG).

2.2.2. NGS-based gene panel

An NGS-based gene panel was obtained from the SNUH Molecular Diagnostics Laboratory (Seoul, Korea). The gene panel comprised 244 candidate genes related to IRD, which were selected from the Retinal Information Network, NEIBank, and RetinaCentral. The tested genes are listed in the Appendix. A total of 244 genes were covered for all coding exons, 5' and 3' UTRs, and each exon flanked by alternative splicing sites. The variant interpretation was performed using a previously reported method [15,16] and following the criteria presented at the 2015 American College of Medical Genetics and Genomics (ACMG) standards and guidelines [17].

2.2.3. Whole exome sequencing

WES was performed, using DNA from the patients, by a commercial service provider (Macrogen Inc., Seoul, Korea). The genomic DNA samples were enriched with the Agilent SureSelect Human All Exon Kit V6 array (Agilent Technologies, Santa Clara, CA, USA) and

sequenced using an Illumina NavaSeq 6000 system (Illumina, San Diego, CA, USA). The sequence reads were aligned to the reference human genome (hg38) using the Burrows-Wheeler Alignment Tool [18]. Reads with a mapping quality score of less than 10 were removed using SAMtools [19]. The Picard MarkDuplicates tool was used to identify and discard read duplicates. Processed variant filtering was performed using the Genome Analysis Toolkit software [20], and SnpEff was used for variant annotation.

Among the WES data, variants from the genes related to IRD in the Retinal Information Network were selected for assessment. The variants were classified according to the 2015 ACMG standards and guidelines [17] using InterVar: pathogenic, likely pathogenic, uncertain significance, likely benign, or benign. Pathogenic and likely pathogenic variants were considered disease-causing mutations (Table 1).

Causative mutations were discovered using the following steps: (1) variants with a minor allele frequency greater than 0.01 in the 1000 Genomes database, Exome Aggregation Consortium database (ExAC), Genome Aggregation database (gnomAD), NHLBI GO Exome Sequencing Project database (ESP), and the database of single nucleotide polymorphisms (dbSNP) were discarded; (2) mutations located in the intron that do not affect the splicing site were sorted out; (3) synonymous variants that did not affect the splicing site

were removed; and (4) in silico analysis using multiple algorithms, including PolyPhen2 [21], SIFT [22], MutationTaster [23], MutationAssessor [24], FATHMM [25], GERP++ [26], PhastCons [27], and PhyloP [28] were used to rule out benign. After the data were sorted, nonsynonymous variants were retained for further analysis.

2.2.4. ACMG guidelines for the interpretation of genetic variants

Richards et al. [17] proposed the guidelines for the interpretation of sequence variants of causative genes that cause the disease in Mendelian inheritance pattern. This guideline classifies the mutations into five categories: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign. This classification is determined based on pathogenic evidence from multiple evaluation categories, such as population data, computational and predictive data, functional studies, and segregation analysis (Figure 2). By combining the type of evidence strength assigned to these evaluation items, one of the five pathogenic grades is determined.

Chapter 3. Results

Overall, 121 patients (86 men and 35 women) from 116 families were involved. The mean age of the study subjects was 6.9 ± 5.6 years at the time when they developed visual symptoms associated with IRD (range, 0–18 years). Strabismus (28.9%, 35/121), decreased vision (39.7%, 48/121), and night blindness (14.9%, 18/121) were common initial symptoms at disease presentation. The median age at the time of genetic tests was 16.7 ± 9.7 years (range, 0–30 years) on average. RP (43.0%, 52/121), X-linked retinoschisis (XRS, 28.1%, 34/121), and Stargardt disease (5.8%, 7/121) were the most common phenotypes in this cohort (Table 2).

Molecular diagnosis was confirmed in 55.4% (67/121) of patients with IRD. The results varied according to the type of test: RS1 gene sequencing (79.4%, 27/34), NGS-based gene panel (45.5%, 20/44), and WES (41.2%, 21/51). Of the 67 patients with genetic confirmation, 10.4% (7/67) had autosomal dominant disorders, 43.3% (29/67) had autosomal recessive diseases, and 46.3% (31/67) had X-linked disorders. The most frequently implicated genes were RS1 (27), EYS (6), ABCA4 (5), and RP1 (5), and a total of 23 genes were identified to be responsible for the diseases (Table 3).

Among the 27 XRS patients, c.286T>C (p.Trp96Arg) (4), c.544C>T (p.Arg182Cys) (3), and c.410T>C (p.Leu137Pro) (3) were identified as common mutations. Three of the four probands with a c.286T>C variant were in the same family; cases #7 and #8 were siblings and the other proband (case #21) was their cousin (Figure 3). The mother of case #21 was a carrier of the c.286T>C mutation, and her father had poor vision since he was young. Cases #10 and #20 are siblings, and they have a c.410T>C variant.

Three patients with Stargardt disease (cases #35, #38, and #41) had a c.880C>T (p.Gln294X) mutation in ABCA4, which was frequently identified in this cohort.

Among the patients with RP, two probands (cases #40 and #66) had a heterozygous c.4957dup (p.Ser1653Lysframeshift) mutation and two patients (cases #55 and #56) had a homozygous c.4957dup (p.Ser1653Lysframeshift) mutation in EYS. Three probands (cases #40, #44, and #63) had a heterozygous c.6557G>A (p.Gly2186Glu) mutation in EYS. RP1 related RP was shown to be inherited in either an autosomal dominant (cases #46 and #61) or recessive manner (cases #43, #52, and #60).

Chapter 4. Discussion

In this study, we performed the genetic analysis of a cohort of 121 Korean children with IRD. Molecular diagnosis was confirmed in 67 probands (55.4%). To the best of our knowledge, this is the first study to evaluate the diagnostic accuracy and causative variants in Korean children with various IRD on a large scale.

IRD is a group of diseases that can cause severe visual deterioration or blindness. It is caused by one or more genetic defects in the retinal function and structures. IRD is one of the leading cause of blindness worldwide. With the recent advances in imaging and genetic diagnostic modalities, the understanding and detection of IRD have been improving.

Several attempts also have been made for the patients with IRD to restore the loss of photoreceptors and/or RPE, including artificial retinal prosthesis [29], gene therapy [30], and stem cell implantation [31-33]. However, there are still many hurdles to overcome for these treatment options, which are not widely applied and only limited to clinical trials. Thus, the management of patients with IRD is still extremely challenging.

Recently, the Food and Drug Administration approved voretigene neparvovec-rzyl (LUXTURNA®, Spark Therapeutics, Inc., Philadelphia, PA, USA), the first gene therapy for

the treatment of patients with biallelic RPE65 mutation-associated retinal dystrophy [11]. Furthermore, more than 30 clinical trials for gene therapy in patients with IRD are ongoing at ClinicalTrials.gov.

Clinical studies for stem cell therapy in IRD are ongoing, and it is now possible to safely deliver stem cell-derived RPEs to the human eye. [10] However, its clinical efficacy is still behind the gene therapy. Both gene therapy and stem cell treatment share the therapeutic goal to restore the diseased genes or cells. Moreover, IRD is caused by genetic abnormalities in the retinal structure and/or function. Therefore, an understanding of the genetic background of IRD may be helpful in the investigation of stem cell treatment.

For the upcoming growth in gene therapy, identification of causative mutations in IRD is necessary for accurate diagnosis and should be preceded by the use of new treatment options. Pediatric IRD patients require long-term care and support, which places a huge burden on their families and on the society. Therefore, it is important to study the pattern of clinical and genetic etiology in childhood-onset IRD. Timely recognition and appropriate management of IRD can have important implications on the health and development of children.

The overall diagnostic yield in this study was 55.4%. Previous studies on pediatric IRD from other groups reported diagnostic yields of 100% (n = 71) from the United Arab Emirates

[34]; 77% (n = 68) from Finland [35]; 78% (n = 59) [36] and 78.8% (n = 85) [37] from the UK. The diagnostic yield in this study was relatively lower than that reported in previous studies. The possible causes for this discrepancy may include differences in patient demographics, including age, ethnicity, and disease entities.

The likelihood of a genetic test clarifying the molecular etiology is related to the pretest probability of subjects with a monogenic disorder, family history, specific clinical presentations, and/or early childhood onset [36]. We included patients less than 30 years of age who had developed visual problems before the age of 18 years and the mean age at which IRD appeared in the study subjects was 6.9 ± 5.6 years. However, the median age at genetic tests was 16.7 ± 9.7 years (range, 0–30 years) on average. Therefore, the age of patients in this study is relatively higher than those from other studies; most patients presented visual symptoms within the first 5 years of life in the study by Khan [34]. Genetic tests were performed for patients under 5 years old in the study by Lenassi et al. [35] and that on patients less than 16 years old in the report by Taylor et al. [36]. Subjects aged 2–18 years were enrolled in the study by Avela et al. [34]. The difference in age distribution in the study group may explain the relatively lower diagnostic rate in this cohort.

Khan suggested a phenotype-guided genetic testing in his study and reported a molecular

diagnostic yield of 100% using this method, which is noteworthy [34]. He selected specific genes for molecular tests in patients with typical clinical and electrophysiological features. Stargardt disease, achromatopsia, and cone-rod dystrophy were common diagnoses, and all the subjects were from consanguineous or endogamous families. Therefore, the pretest probability of genetic analysis may be much higher than that of the other groups. We also achieved a higher diagnostic rate in patients with XRS (79.4%). This result suggests that it is important to conduct a clinical diagnosis as much as possible by a thorough evaluation of patients and perform specific genetic tests based on the clinical characteristics of the subjects.

Three patients with Stargardt disease had a c.880C>T (p.Gln294X) mutation in ABCA4, and these variants were commonly discovered in this cohort. This mutation was recently reported as a novel disease-causing mutation in other Korean patients [38,39].

The mutations c.4957dup (p.Ser1653Lysframeshift) and c.6557G>A (p.Gly2186Glu) in EYS have been frequently identified in RP patients in East Asian populations [40-44]. These causative variants were also common in this study population. These results suggest the possibility that these mutations in EYS originated from common ancestors and spread throughout East Asia.

This study had a retrospective design, and our data may have been affected by selection

bias. However, this study was conducted at a tertiary referral center and enrolled subjects consecutively. Therefore, we collected crude data on the distribution of genetic and clinical features of pediatric patients with IRD in the Korean population. Similar to recently published reports from international and multicenter groups [45], further nationwide or population-based studies from similar ethnicities are necessary to obtain detailed epidemiologic data on child-onset IRD.

In conclusion, we conducted a genetic analysis of Korean pediatric patients with IRD on a large scale and discovered genetic characteristics in this cohort. The results from our study will serve as a basis for genetic counseling of IRD patients and their family members and lay a cornerstone for the progress of the regenerative medicine in the future.

Appendix

A total of 244 genes tested using next-generation sequencing-based gene panel are listed below.

ABCA4, ABCC6, ABHD12, ACO2, ADAM9, ADAMTS18, ADIPOR1, AGBL5, AHI1, AHR, AIPL1, ALMS1, ARHGEF18, ARL2BP, ARL3, ARL6, ATF6, ATXN7, BBIP1, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, BEST1, C12orf65, C1QTNF5, C21orf2, C2orf71, C8orf37, CA4, CABP4, CACNA1F, CACNA2D4, CAPN5, CC2D2A, CDH23, CDH3, CDHR1, CEP164, CEP250, CEP290, CERKL, CFH, CHM, CIB2, CLCC1, CLN3, CLRN1, CNGA1, CNGA3, CNGB1, CNGB3, CNNM4, COL11A1, COL2A1, COL9A1, CRB1, CRX, CSPP1, CTNNA1, CYP4V2, DFNB31, DHDDS, DHX38, DMD, DRAM2, EFEMP1, ELOVL1, ESPN, EYS, FAM161A, FLVCR1, FSCN2, FZD4, GDF6, GNAT1, GNAT2, GNB3, GNPTG, GPR125, GPR179, GPR98, GRK1, GRM6, GUCA1A, GUCA1B, GUCY2D, HARS, HGSNAT, HK1, HMCN1, HMX1, IDH3B, IFT140, IFT172, IFT27, IFT81, IMPDH1, IMPG1, IMPG2, INPP5E, INVS, IQCB1, ITM2B, JAG1, KCNJ13, KCNV2, KIAA0090, KIAA1549, KIF11, KLHL7, LAMA1, LCA5, LRAT, LRIT3, LRP5, LZTFL1, MAK, MAPKAPK3, MERTK, MFN2, MFRP, MFSD8, MKKS, MKS1, MTTP, MVK, MYO7A, NDP, NEK2, NEUROD1, NMNAT1, NPHP1, NPHP3, NPHP4, NR2E3, NRL, NYX, OAT, OFD1,

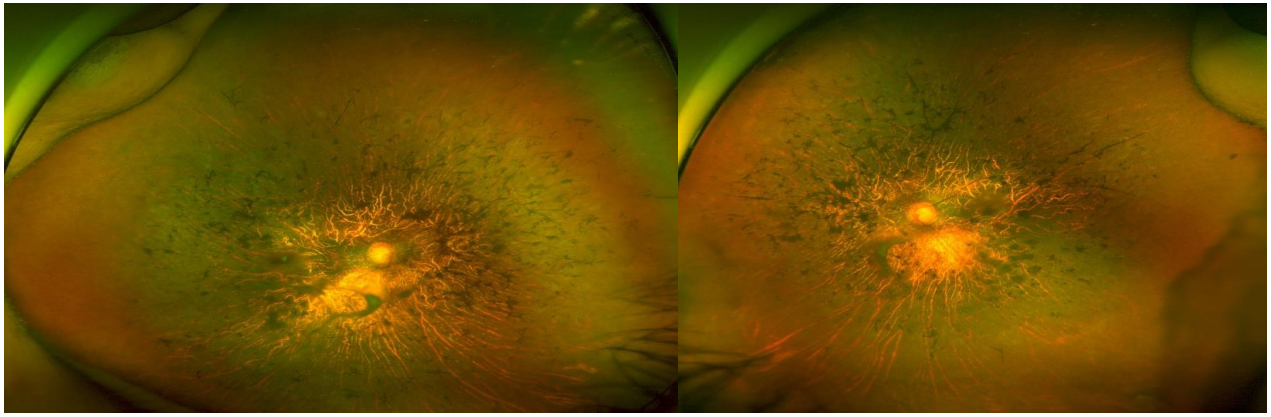
OPA3, OPN1LW, OPN1MW, OTX2, PANK2, PAX2, PCDH15, PCYT1A, PDE6A, PDE6B, PDE6C, PDE6G, PDE6H, PDZD7, PEX1, PEX2, PEX7, PGK1, PHYH, PITPNM3, PLA2G5, PLK1S1, PLK4, PNPLA6, POC1B, POC5, POMGNT1, PRCD, PROM1, PRPF3, PRPF31, PRPF4, PRPF6, PRPF8, PRPH2, PRPS1, RAB28, RAX2, RB1, RBP3, RBP4, RD3, RDH11, RDH12, RDH5, REEP6, RGR, RGS9, RGS9BP, RHO, RIMS1, RLBP1, ROM1, RP1, RP1L1, RP2, RP9, RPE65, RPGR, RPGRIP1, RPGRIP1L, RS1, SAG, SAMD11, SDCCAG8, SEMA4A, SLC24A1, SLC25A46, SLC7A14, SNRNP200, SPATA7, SPP2, TEAD1, TIMP3, TMEM216, TMEM237, TOPORS, TREX1, TRIM32, TRNT1, TRPM1, TSPAN12, TTC8, TTLL5, TTPA, TUB, TUBGCP4, TUBGCP6, TULP1, UNC119, USH1C, USH1G, USH2A, VCAN, WDPCP, WDR19, WFS1, ZNF408, ZNF423, ZNF513

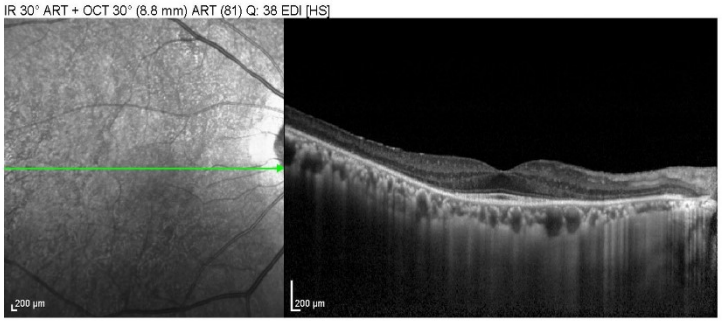
Figure 1. Illustrative images of patients with inherited retinal disease.

Fundus photos show diffuse retinal pigmentary abnormalities on the retinas of both eyes (top).

Optical coherence tomography images show the loss and disruption of outer retinal tissues, indicated by red dotted lines (second row).

A visual field defect is seen (third row), and the electrical response to light is severely constricted, as shown in the electroretinogram (bottom).

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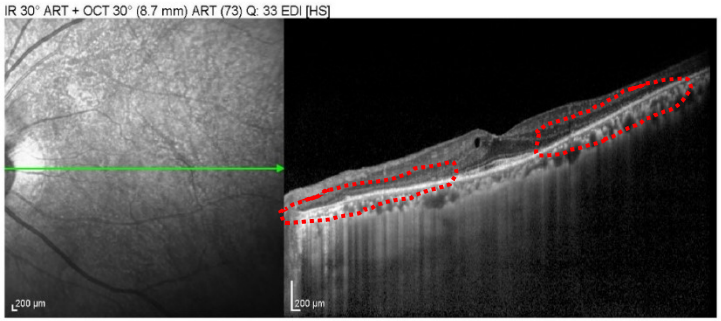
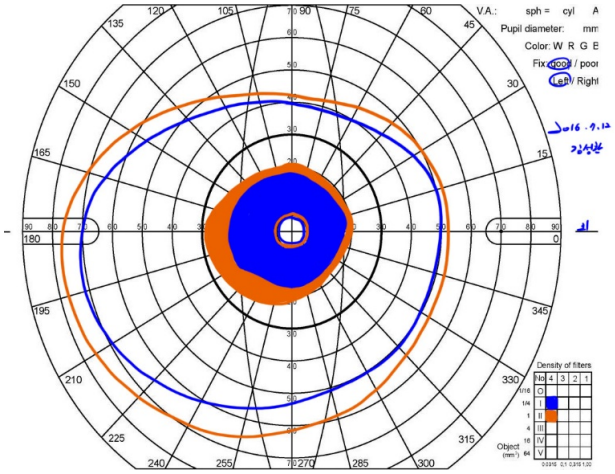
200 µm
200 µm


IR 30° ART + OCT 30° (8.7 mm) ART (73) Q: 33 EDI [HS]
200 µm
200 µm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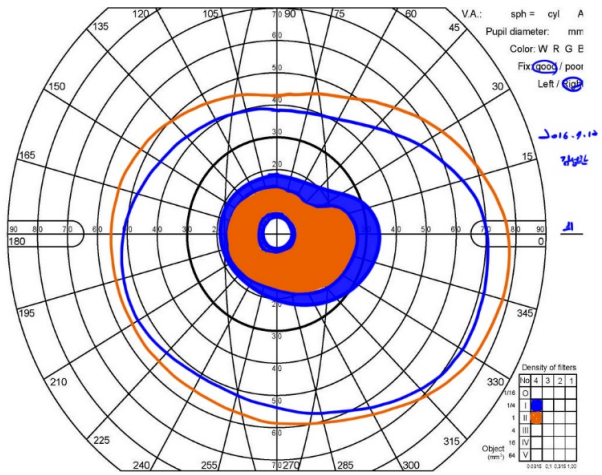
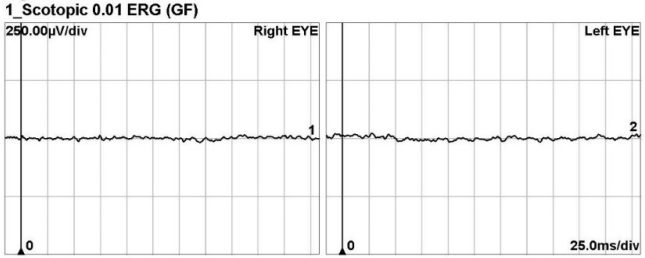
Pupil diameter: mm
Color: W R G E
Fix: good / poor
Left / Right
Density of filters
No 4 3 2 1
Object (mm²) 64


V.A.: sph = cyl A
Pupil diameter: mm
Color: W R G E
Fix: good / poor
Left / Right
Density of filters
No 4 3 2 1
Object (mm²) 64

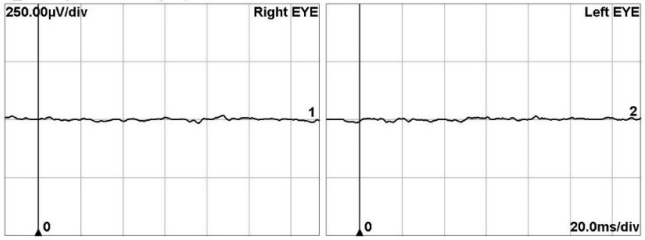
Diagnosis:

1_Scotopic 0.01 ERG (GF)



Channel	a [ms]	b [ms]	a-wave	b-wave	b/a
1 R-1	0.0	0.0			
2 L-2	0.0	0.0			
Normals					
1 R-1	-	-			

2_Scotopic 3.0 ERG (GF)



Normals	-	-			
Channel	a [ms]	b [ms]	a-wave	b-wave	b/a
1 R-1	0.0	0.0			
2 L-2	0.0	0.0			

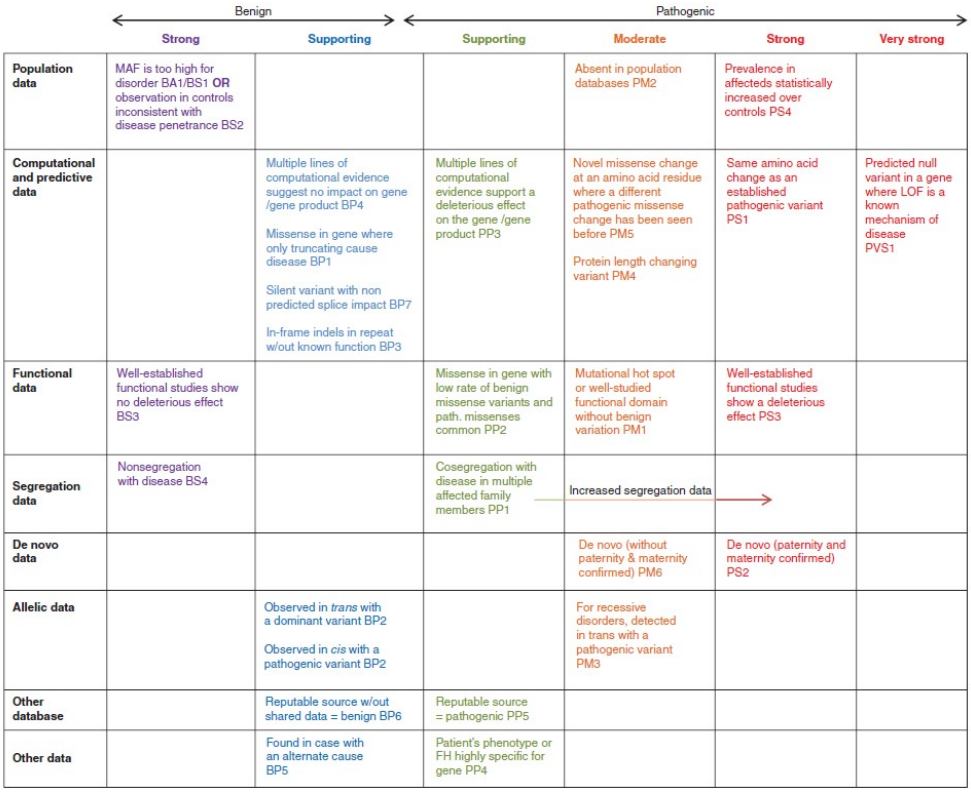
6_Photopic 3.0 Flicker 30Hz ERG (GF)

Normals	59-66	34.0µV-173µV
Channel	**P1 [ms]**	**N1 - P1**
1 R-1	41.7 (!)	5.27µV (!)
2 L-2	40.5 (!)	5.74µV (!)

Table 1. Combination table for genetic variants classified as pathogenic and likely pathogenic from the ACMG standards and guidelines [17].

Pathogenic	(i) 1 Very strong (PVS1) *AND* (a) ≥1 Strong (PS1–PS4) *OR* (b) ≥2 Moderate (PM1–PM6) *OR* (c) 1 Moderate (PM1–PM6) *AND* 1 supporting (PP1–PP5) *OR* (d) ≥2 Supporting (PP1–PP5) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 1 Strong (PS1–PS4) *AND* (a) ≥3 Moderate (PM1–PM6) *OR* (b) 2 Moderate (PM1–PM6) *AND* ≥ 2 Supporting (PP1–PP5) *OR* (c) 1 Moderate (PM1–PM6) AND ≥ 4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)

Figure 2. Evidence framework from the ACMG standards and guidelines [17].



	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

BS, benign strong; BP, benign supporting; FH, family history; LOF, loss of function;

MAF, minor allele frequency; path., pathogenic; PM, pathogenic moderate;

PP, pathogenic supporting; PS, pathogenic strong; PVS, pathogenic very strong.

Table 2. Clinical diagnoses and demographics of the patients with inherited retinal diseases (n = 121).

Clinical diagnoses of the patients were classified into three categories: photoreceptor disease, macular disease, and third-branch disorders. This classification system was followed as in the study by Stone et al. [46].

`	All Children (n = 121)		Families (n = 116)	Males (n = 86)	Females (n = 35)
Photoreceptor Diseases	70 (100.0%)	57.9%	68	44	26
RP	54 (77.1%)	44.6%	52	35	19
Usher syndrome	4 (5.7%)	3.3%	4	1	3
CD, CRD	5 (7.1%)	4.1%	5	4	1
LCA	4 (5.7%)	3.3%	4	2	2
CSNB	2 (2.9%)	1.7%	2	1	1
Retinitis punctata albescens	1 (1.4%)	0.8%	1	1	0
Macular Diseases	15 (100.0%)	12.4%	15	6	9
STGD	7 (46.7%)	5.8%	7	3	4
MD	5 (33.3%)	4.1%	5	2	3
Best disease	3 (20.0%)	2.5%	3	1	2
Third Branch Disorders	36 (100.0%)	29.8%	33	36	0
RS	34 (94.4%)	28.1%	31	34	0
CDM	2 (5.6%)	1.7%	2	2	0

Abbreviations: RP, retinitis pigmentosa; CD, cone dystrophy; CRD, cone-rod dystrophy. LCA, Leber congenital amaurosis; CSNB, congenital stationary night blindness; STGD, Stargardt disease; MD, macular dystrophy; RS, retinoschisis; CDM, choroideremia.

Table 3. The results of the genetic analyses of the patients with known causative mutations.

Case	Phenotype	Causative Gene	Inheritance Pattern	Zygosity	Mutation Type	cDNA Change	Protein Change	Type of Genetic Study
1	RS	*RS1*	X-linked	Hemi	Missense	c.544C>T	p.R182C	Direct gene sequencing
2	RS	*RS1*	X-linked	Hemi	Missense	c.590G>A	p.R197H	Direct gene sequencing
3	RS	*RS1*	X-linked	Hemi	Missense	c.544C>T	p.R182C	Direct gene sequencing
4	RS	*RS1*	X-linked	Hemi	Missense	c.214G>A	p.E72K	Direct gene sequencing
5	RS	*RS1*	X-linked	Hemi	Missense	c.464A>G	p.Y155C	Direct gene sequencing
6	RS	*RS1*	X-linked	Hemi	Missense	c.647T>C	p.L216P	Direct gene sequencing
7	RS	*RS1*	X-linked	Hemi	Missense	c.286T>C	p.W96R	Direct gene sequencing
8	RS	*RS1*	X-linked	Hemi	Missense	c.286T>C	p.W96R	Direct gene sequencing
9	RS	*RS1*	X-linked	Hemi	Missense	c.544C>T	p.R182C	Direct gene sequencing
10	RS	*RS1*	X-linked	Hemi	Missense	c.410T>C	p.L137P	Direct gene sequencing
11	RS	*RS1*	X-linked	Hemi	Missense	c.638G>A	p.R213Q	Direct gene sequencing
12	RS	*RS1*	X-linked	Hemi	Missense	c.422G>A	p.R141H	Direct gene sequencing
13	RS	*RS1*	X-linked	Hemi	Missense	c.647T>C	p.L216P	Direct gene sequencing
14	RS	*RS1*	X-linked	Hemi	Splicing	c.185-1G>A	IVS3	Direct gene sequencing
15	RS	*RS1*	X-linked	Hemi	Missense	c.214G>A	p.E72K	Direct gene sequencing
16	RS	*RS1*	X-linked	Hemi	Missense	c.422G>A	p.R141H	Direct gene sequencing
17	RS	*RS1*	X-linked	Hemi	Missense	c.404G>A	p.G135E	Direct gene sequencing
18	RS	*RS1*	X-linked	Hemi	Missense	c.410T>C	p.L137P	Direct gene sequencing
19	RS	*RS1*	X-linked	Hemi	Missense	c.589C>T	p.R197C	Direct gene sequencing
20	RS	*RS1*	X-linked	Hemi	Missense	c.410T>C	p.L137P	Direct gene sequencing
21	RS	*RS1*	X-linked	Hemi	Missense	c.286T>C	p.W96R	Direct gene sequencing
22	RS	*RS1*	X-linked	Hemi	Missense	c.574C>T	p.P192S	Direct gene sequencing
23	RS	*RS1*	X-linked	Hemi	Missense	c.599G>A	p.R200H	Direct gene sequencing
24	RS	*RS1*	X-linked	Hemi	In frame duplication	c.306_308dupGCT	p.L103dup	Direct gene sequencing
25	RS	*RS1*	X-linked	Hemi	Missense	c.286T>C	p.W96R	Direct gene sequencing
26	RS	*RS1*	X-linked	Hemi	Missense	c.499A>G	p.K167E	Direct gene sequencing
27	RS	*RS1*	X-linked	Hemi	Missense	c.625C>T	p.R209C	Direct gene sequencing
28	RP	*RP2*	X-linked	Hemi	Splicing	c.102+2T>C	.	NGS-based gene panel & Whole exome sequencing
29	Best	*BEST1*	AR	Het	Missense	c.584C>T	p.A195V	NGS-based gene panel
					Missense	c.632T>C	p.L211P	
30	Usher	*MYO7A*	AR	Het	Nonsense	c.52C>T	p.Q18*	NGS-based gene panel
					Splicing	c.3503+2T>G	.	

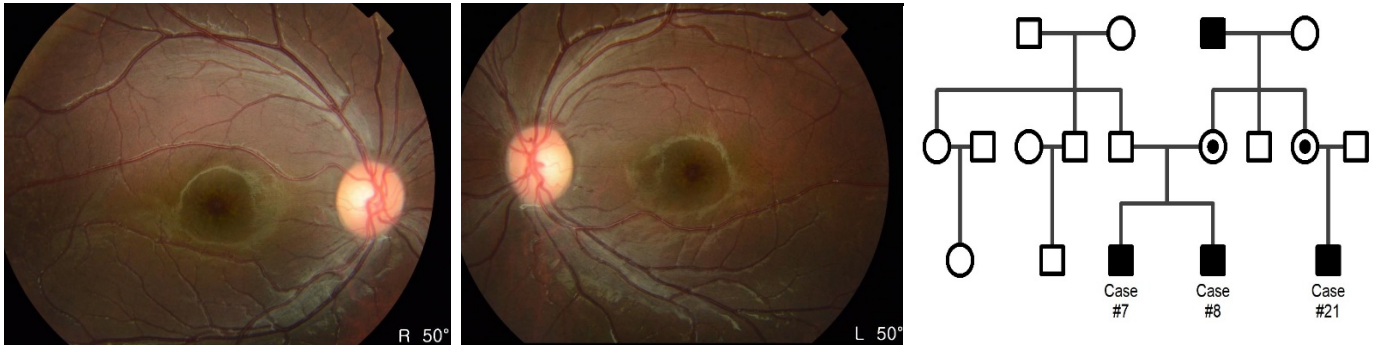
31	CRD	*GUCY20*	AD	Het	Missense	c.2513G>A	p.R838H	NGS-based gene panel
32	RP	*TULP1*	AR	Hom	Missense	c.1145T>C	p.F382S	NGS-based gene panel
33	STGD	*ABCA4*	AR	Het	Splicing	c.1760+2T>G	p.?	NGS-based gene panel
					Missense	c.1699G>A	p.V567Met	
34	CSNB	*CGNB1*	AR	Het	Nonsense	c.2977-1G>A	p.?	NGS-based gene panel
				Het	Nonsense	c.217+5G>C	p.?	
35	STGD	*ABCA4*	AR	Het	Nonsense	c.880C>T	p.Q294*	NGS-based gene panel
					Missense	c.6050G>A	p.C2017Y	
36	RP	*PRPF31*	AD	Het	Frameshift	c.914_931delins CCAGTGT	p.V305Afs*15	NGS-based gene panel
37	RP	*RP2*	X-linked	Hemi	Frameshift	c.385_386del	p.L129Vfs*9	NGS-based gene panel
38	STGD	*ABCA4*	AR	Het	Nonsense	c.880C>T	p.Q294*	NGS-based gene panel
					Missense	c.4748T>C	p.L1583P	
39	CSNB	*WM1*	AR	Het	Frameshift	c.675_677delinsC	p.K225Nfs*34	NGS-based gene panel
					Missense	c.2783G>A	p.R928Q	
40	RP	*EYS*	AR	Het	Frameshift	c.4957dup	p.S1653KfsTer2	NGS-based gene panel
					Missense	c.6557G>A	p.G2186E	
41	STGD	*ABCA4*	AR	Het	Nonsense	c.880C>T	c.880C>T	NGS-based gene panel
					Missense	c.6563T>C	p.F2188S	
42	LCA	*CRB1*	AR	Het	Nonsense	c.1576C>T	p.R526*	NGS-based gene panel
					Missense	c.998G>A	p.G333D	
43	RP	*RP1*	AR	Hom	Frameshift	c.6181delA	p.I2061Sfs*12	NGS-based gene panel
44	RP	*EYS*	AR	Het	Frameshift	c.4245_4246del	p.Q1415Hfs*14	NGS-based gene panel
					Missense	c.6557G>A	p.G2186E	
45	STGD	*ABAC4*	AR	Het	Frameshift	c.6146delA	p.K2049Rfs*12	NGS-based gene panel
					Missense	c.3349A>G	p.T1117A	
46	RP	*RP1*	AD	Het	Nonsense	c.2143C>T	p.Q715*	NGS-based gene panel
47	Retinitis punctate albescens	*TULP1*	AR	Het	Missense	c.931C>T	p.R311W	NGS-based gene panel
					Missense	c.349G>A	p.E117K	
48	RP	*IMPDH1*	AD	Het	Missense	c.A968T	p.K323M	Whole exome sequencing
49	LCA	*RDH12*	AR	Het	Missense	c.C377T	p.A126V	Whole exome sequencing
					Missense	c.C715G	p.R239G	
50	CDM	*CHM*	X-linked	Hemi	Frameshift	c.525_526del	p.T175fs	Whole exome sequencing
51	RP	*KIF11*	AD	Het	Frameshift	c.2514_2518delTGAAA	p.N838fs	Whole exome sequencing
52	RP	*RP1*	AR	Het	Frameshift	c.4196del	p.C1399fs	Whole exome sequencing
					Nonsense	c.5971C>T	p.Q1991*	
53	RP	*BBS10*	AR	Het	Nonsense	c.1391C>G	p.S464*	Whole exome sequencing

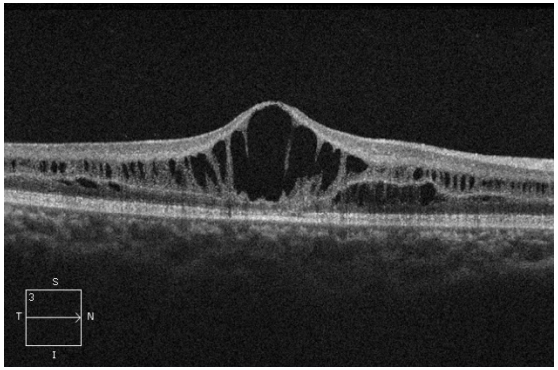
					Frameshift	c.365dupA	p.N122fs	
54	RP	*RP2*	X-linked	Hemi	Missense	c.352C>T	p.R118C	Whole exome sequencing
55	RP	*EYS*	AR	Hom	Frameshift	c.4957dupA	p.S1653fs	Whole exome sequencing
56	RP	*EYS*	AR	Hom	Frameshift	c.4957dupA	p.S1653fs	Whole exome sequencing
57	RP	*TULP1*	AR	Het	Nonsense	c.25C>T	p.R9*	Whole exome sequencing
					Missense	c.349G>A	p.E117K	
58	RP	*NR2E3*	AD	Het	Missense	c.166G>A	p.G56R	Whole exome sequencing
59	CRD	*RAB28*	AR	Hom	Missense	c.68C>T	p.S23F	Whole exome sequencing
60	RP	*RP1*	AR	Hom	Frameshift	c.796_797delCA	p.H266fs	Whole exome sequencing
61	RP	*RP1*	AD	Het	Frameshift	c.6181delA	p.I2061fs	Whole exome sequencing
62	RP	*RPGR*	X-linked	Hemi	Nonsense	c.808C>T	p.Q270*	Whole exome sequencing
63	RP	*EYS*	AR	Het	Nonsense	c.8868C>A	p.Y2956*	Whole exome sequencing
					Missense	c.6557G>A	p.G2186E	
64	RP	*BBS10*	AR	Het	Frameshift	c.365dupA	p.N122fs	Whole exome sequencing
					Missense	c.431T>C	p.I144T	
65	LCA	*NMNAT1*	AR	Het	Missense	c.709C>T	p.R237C	Whole exome sequencing
					Missense	c.703A>G	p.S235G	
66	RP	*EYS*	AR	Het	Frameshift	c.4957dupA	p.S1653fs	Whole exome sequencing
					Frameshift	c.9431delA	p.N3144fs	
67	RP	*MERTK*	AR	Hom	Frameshift	c.225delA	p.G76fs	Whole exome sequencing

Abbreviations: Hemi, hemizygous; Het, heterozygous; Hom, homozygous; AD, autosomal dominant; AR, autosomal recessive; RS, retinoschisis; RP, retinitis pigmentosa; Best, Best disease; Usher syndrome; CRD, cone-rod dystrophy; STGD, Stargardt disease. CSNB, congenital stationary night blindness; LCA, Leber congenital amaurosis; CDM, choroideremia; NGS, next-generation sequencing.

Figure 3. Representative cases of three X-linked retinoschisis patients in the same family.

Color fundus image shows spoke wheel appearance at the fovea in both eyes of Case #7, suggesting retinoschisis. Spectral domain optical coherence tomography reveals retinoschisis in both eyes. Full-field electroretinogram demonstrates reduced b wave and an electronegative waveform in mixed rod and cone response. Case #7 and #8 had a c.286T>C variant in *RS1* and they are siblings. The other proband (case #21) is their cousin and his mother is a carrier for a c.286T>C mutation. Their grandfather had poor vision since he was young.

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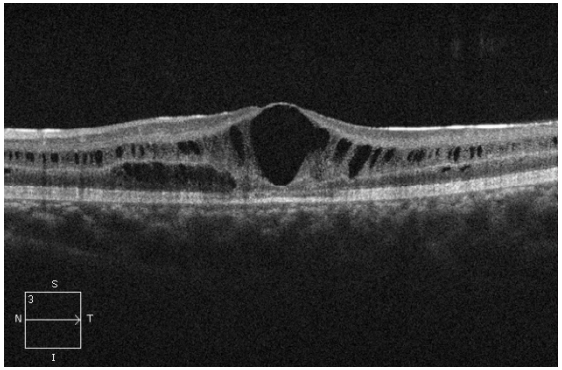
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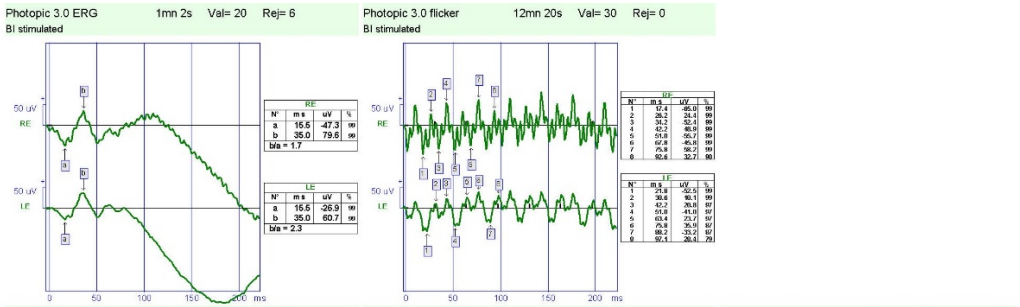
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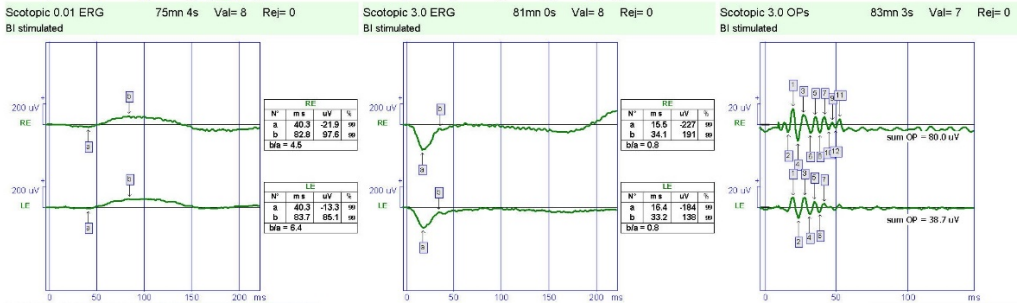
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국문 초록

한국인 소아 유전성 망막질환 환자의 유전 분석

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배기웅

유전성 망막질환(inherited retinal disease; IRD)은 다양한 임상양상과 유전적 원인을 가진 일련의 질환을 말하며, 주로 단일 유전자 이상에 의해 발생하는 것으로 알려져 있다. 유전성 망막질환은 심각한 시력 손실 혹은 실명을 초래할 수 있으므로 적절한 치료 및 관리를 위해, 정확하고 신속한 진단이 요구된다.

안구는 외부에 노출되어 있어 비교적 쉽게 검진 및 처치를 위해 접근할 수 있으며, 면역 관용 구역(immune-privileged space)으로서 이식 거부 반응의 가능성이 낮다. 뿐만 아니라, 상대적으로 크기가 작고 분리된 구획이기에 치료 효과를 거두기 위해 소량의 약제나 세포를 필요로 한다는 점에서 줄기세포 치료나 유전자 치료를 비롯한 재생 의학의 좋은 표적 기관이 된다. 이에 유전성

망막질환에 대한 줄기세포 치료 및 유전자 치료 등 근본적인 치료에 대한 연구가 활발하게 진행 중이다.

최근 RPE65 유전자 이상에 의해 발생한 망막이영양증 (retinal dystrophy)에 대한 유전자 치료제로 voretigene neparvovec-rzyl (LUXTURNA®)이 미국 식품의약국 (FDA)에 승인 받았다. 그리고 황반변성 환자의 섬유아세포로부터 역분화 줄기세포를 만들고 이로부터 망막색소상피세포를 분화시켜 다시 그 환자에게 자가 줄기세포를 성공적으로 이식하였다는 보고가 있다. 이 연구를 통해, 줄기세포 치료의 안전성은 입증되었지만 시력 개선은 확인되지 않아서 줄기세포 치료가 임상에 널리 적용되기에는 지속적인 연구 및 개발이 필요한 실정이다.

줄기세포 치료와 유전자 치료는 세포 혹은 유전자 이상으로 인해 발생하는 질환을 치료한다는 공통점을 가지고 있다. 특히, 유전성 망막질환은 망막의 구조 및 기능과 관련된 유전자의 이상에 의해 발생하므로 그 유전자 이상에 대해 분석하고 연구하는 것은 줄기세포 치료에 대한 이해를 높이는데 기여할 수 있을 것으로 사료된다. 이에 본 연구를 통해, 한국인 소아 유전성

망막질환 환자를 대상으로 유전적 원인 및 임상양상에 대해 살펴 보았다.

2011년부터 2020년까지 서울대학교병원 유전성 망막질환 클리닉을 방문한 소아 환자 121명의 진료 기록을 후향적으로 분석하였고, 원인 유전자 규명을 위해 단일유전자 검사(targeted single gene sequencing), 차세대 염기서열 분석 기반 기반 유전자 패널 검사(next-generation sequencing based gene panel), 또는 전장엑솜시퀀싱 검사(whole exome sequencing)를 적용하였다.

총 116 가계로부터 남성 86명, 여성 35명의 소아 유전성 망막질환 환자가 포함되었고, 환자들은 평균적으로 6.9±5.6세(범위, 0-18 세)에 야맹증, 시력 저하 등의 유전성 망막질환과 관련된 시각 증상을 호소하였다. 망막색소변성(43.0%), X 염색체관련 망막층간분리(28.1%), 스타가르트병(5.8%) 등이 본 연구집단에서 흔히 발견된 유전성 망막질환이었다.

모든 피험자 중 67명(55.4%)에서 유전성 망막질환의 유전적 원인이 규명되었고, 총 23개의 원인 유전자가 확인되었다. 본 연구의 결과는 유전성

망막질환 환자와 가족에 대한 진료에 있어서, 역학(epidemiology)적인 측면에서 주된 참고자료가 될 것이다.

주요어 : 유전성 망막질환, 망막색소변성, 유전자 패널 검사, 차세대 염기서열 분석, 전장엑솜시퀀싱

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