Phenotypic and Genotypic Characterization of Patients with Retinitis Pigmentosa in a Tertiary Hospital in the Philippines

Tamilyn Chelsea C. Laddaran, MD, DPBO[1], Manuel Benjamin B. Ibanez IV, MD, DPBO[1], Marianne Grace P. Navarrete, MD[1]

[1]Department of Ophthalmology, Makati Medical Center, Makati, Philippines

Correspondence: Tamilyn Chelsea C. Laddaran, MD, DPBO
Office Address: Department of Ophthalmology, Makati Medical Center, 2 Amorsolo Street, Legaspi Village, Makati City, Philippines
Office Phone Number: +639985377798
Email Address: tamiladdaran@gmail.com

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ABSTRACT

Objectives: To determine the phenotypic and genotypic characterization of individuals with retinitis pigmentosa (RP), identify their genetic etiologies, and provide counseling to affected patients.

Methods: This non-interventional, observational study evaluated 18 patients with clinically-diagnosed RP from 15 different families. The patients underwent complete ophthalmological examination with retinal functional and morphologic assessment. Genetic testing was done using next-generation sequencing.

Results: Ten gene mutations with 22 variants were identified. The inheritance pattern was predominantly autosomal recessive (70%). The most common mutation was EYS (27.8%). One possible novel gene, RGS7, and novel variants of CNGB1 were identified. Characteristic RP profiles were observed, with syndromic findings noted in USH2A and BBS5 mutations.

Conclusion: Phenotypic characteristics among different gene mutations have distinct features. This is the first study in the country to demonstrate the genotypic heterogeneity of RP, displaying 22 variants with 3 noted novel mutations.

Keywords: Retinitis pigmentosa, Philippines, genotype, phenotype

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Retinitis pigmentosa (RP) is a heterogeneous, hereditary, retinal degeneration disorder caused by the progressive loss of photoreceptor cells. The typical presentation of the disease includes night blindness, loss of peripheral vision, blurring of vision, and degenerative changes in the retina. It is estimated to affect 1 in 3500 to 5000 people worldwide.[1] Currently, there are no available studies on epidemiology of RP in the Philippines.

A 2014 study conducted at the Department of Health (DOH) Eye Center investigated the etiologies of legal blindness among patients and found that 17 out of 146 (12%) patients had hereditary retinal disorders such as RP and macular dystrophy. Only a few genetic studies in eye diseases have been conducted in the Philippines. A 2003 study at the Philippine General Hospital performed genotype analysis of 5 families with suspected RP, focusing only on the *RHO* and *RDS*/peripherin genes using restriction endonuclease studies.[3] All 5 families tested negative for these mutations, potentially missing causative genes, as there are 54 known genes associated with RP.[4] This study aimed to expand the genetic analysis by employing larger gene panel using next-generation sequencing to comprehensively identify the genetic causes and phenotypes of RP in a Filipino cohort.

METHODS

This was a non-interventional, cross-sectional, descriptive study. This study received ethics review board approval and conformed to the tenets of the Declaration of Helsinki. The study recruited consecutive patients with clinically-diagnosed RP in a tertiary hospital in Manila. Informed consent was obtained. Pretest genetic counseling was provided by an ocular geneticist during the informed consent process. Genetic testing for patients was performed using an inherited retinal dystrophy panel or whole exome sequencing (WES). Patients who refused to undergo ophthalmic exam or provide DNA samples were excluded from the study.

The study participants underwent a detailed clinical history-taking wherein the following information were collected: age, history of ocular symptoms related to the RP, family history, review of systems, and prior ophthalmologic consultations. A family pedigree was made, which required the ages, ethnic backgrounds, diseases, and biological relationships between individuals in their family.

The study participants underwent a routine eye examination, which included visual acuity testing using the standard Early Treatment for Diabetic Retinopathy Study (EDTRS) chart, color vision testing using Ishihara pseudoisochromatic plates, slit-lamp examination, and dilated fundus exam. Diagnostic imaging procedures including colored fundus photography, fundus autofluorescence, and ocular coherence tomography (OCT) scans of the macula were done. Patients who had previously undergone these ocular tests were asked to provide the investigators with copies of their test results. T.C.L. performed the clinical history taking and ocular examination, which were validated by M.B.I.

Ten milliliters (10 ml) of blood samples were extracted and were shipped out for genetic testing using whole exome sequencing. Patients who had previously undergone genetic testing were allowed to forgo this step if they were able to provide their test results. M.B.I. interpreted the genetic testing results. The results were then relayed and explained to the participants.

Statistical Analysis

Data was encoded on MS Excel (Microsoft Corporation, Washington, USA) and analyzed using STATA15 (StataCorp LLC, College Station, TX). Descriptive statistics, such as median and range, were used to present continuous variables while frequency and percentage were used to present categorical data.

RESULTS

A. Clinical Characteristics of Patients with RP

Eighteen (18) Filipino patients from 15 different families were included in the study. **Table 1** shows the summary of demographic and clinical profile of patients with RP included in the study. Seven (7) were male (38.9%) and 11 were female (61.1%). The median age of symptom onset was 13

years old (range 2-50). The most common first symptom noted was night blindness (77.8%, N=14), followed by blurring of vision (22.2%, N=4). The median best-corrected visual acuity (BCVA) in both eyes was 0.7 (range 0-3). Color vision was normal in 50% (N=9), impaired in 44.4% (N=8) and could not be assessed in 1 (5.6%). There were 7 (38.9%) patients who had clear lenses, while 8 (44.5%) were pseudophakic. One (5.56%) had combined nuclear sclerotic cataracts, another 1 (5.56%) had posterior subcapsular cataracts, and 1 (5.56%) was pseudophakic in 1 eye and had a posterior subcapsular cataract in the other eye.

Fundus findings of 100% (N=18) of patients showed bone-spicule pigmentation, 72.2% (N=13) had arteriolar attenuation, 16.7% (N=3) had disc pallor, and 11.1% (N=2) had peripapillary atrophy. On fluorescein angiography, 88.9% (N=16) had a hyperfluorescent ring at the parafovea, 88.9% (N=16) had patchy hypofluorescence corresponding to areas of retinal atrophy throughout the retina, while 11.1% (N=2) had confluent areas of hypofluorescence. On OCT scans of the macula, 55.6% (N=10) of patients had loss of ellipsoid zone centrally, while 33.3% (N=6) had loss of the ellipsoid zone peripherally. Two patients (11.1%) could not be tested with the OCT machine due to inability to focus and poor signal strength. Six patients (33.3%) had hyperreflective foci in the outer retinal layer, corresponding to debris of photoreceptors, 16.7% (N=3) had an epiretinal membrane, while 11.1% (N=2) had hyporeflective cystic spaces in the outer retinal layers corresponding to cystoid macular edema. There was 1 patient (5.56%) with vitreomacular traction and another 1 (5.56%) with choroidal neovascularization (**Table 1**).

B. Identification of Genetic Mutations in Patients with RP

Genetic testing revealed mutations in the *EYS* gene in 5 patients (5 families), *RPGR* gene in 1 patient, *USH2A* gene in 2 patients (2 families), *FAM161A* gene in 2 patients (1 family), *FLVCR1* gene in 1 patient, *RGS7* gene in 2 patients (1 family), *CNGB1* gene in 2 patients (1 family), *BBS5* gene in 1 patient, *PDE6A* gene in 1 patient, and *SPP2* gene in 1 patient (**Figure 1**).

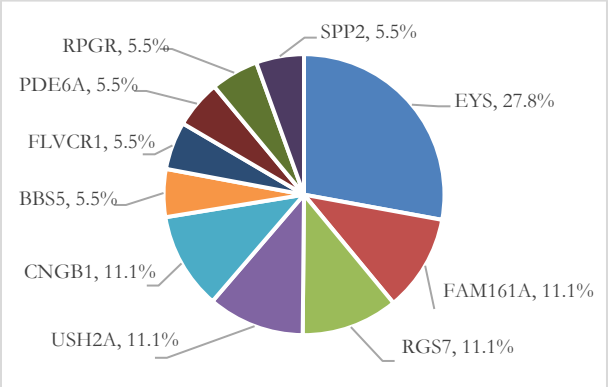

Figure 1. Distribution of genetic mutations

Among the 10 genes found in the study, 70% had an autosomal recessive inheritance, followed by X-linked recessive, autosomal dominant, and unknown inheritance at 10% each (**Table 2**).

C. Genotypic and Phenotypic Correlation

Among the 5 patients with *EYS* gene mutations, the median age of symptom onset was 13 (range 15-20) (**Table 1**). The most common first symptom noted was night blindness (60%, N=3), followed by blurring of vision (40%, N=2). Most of the patients were female at 80% (N=4), and 20% (N=1) were male. The median BCVA on the right was 1 (range 0.4-3.0) and on the left was 0.8 (range 0.4-3.0). One of the patients, PH017, had no light perception on the left eye. Majority had impaired color vision (60%, N=3) while 40% (N=2) had normal color vision. From 5 patients, 7 *EYS* mutations were found, 40% (N=2) of which were missense, 40% (N=2) were nonsense, and 20% was frameshift/nonsense. Out of these 7 variants, 6 were pathogenic (85.7%) and 1 was a variant of unknown significance (VUS) (14.3%). All of the patients presented with bone-spicule pigmentation and arteriolar attenuation, but only 2 patients presented with disc pallor (40%) and 1 with peripapillary atrophy (20%). Three (60%) had patchy hypofluorescence in the retina, 2 (40%) had hyperfluorescent ring in the parafovea, and 2 (40%) had confluent areas of hypofluorescence. Four (80%) had loss of the ellipsoid zone centrally, 2 (40%) had epiretinal membrane, 1 (20%) each had cystoid macular edema and choroidal neovascularization (**Figure 2**). PH017 was unable to focus for OCT measurement.

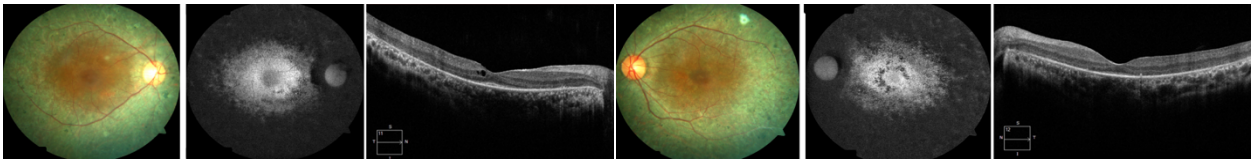

Figure 2. Representative images from PH008, who has compound heterozygous missense *EYS* mutations showing characteristic bone-spicule pigmentation and arteriolar attenuation on colored fundus photo, parafoveal hyperfluorescence on fundus autofluorescence, and loss of ellipsoid zone loss on OCT.

Table 1. Clinical Profile of Filipino Patients with RP

Gene Mutation		*BBS5*	*CNGB1*	*EYS*	*FAM161A*	*FLVCR*	*PDE6A*	*RGS7*	*RPGR*	*SPP2*	*USH2A*
N		1	2	5	2	1	1	2	1	1	2
Mean age at first symptoms (Range)		2 (2)	10.5 (10 – 11)	13 (5 – 20)	10 (7 – 13)	25	8	35 (30 – 40)	10	13	31.5 (13 – 50)
First Symptom, n(%)	Blurring of vision	1 (100)	-	2 (40)	-	-	-	-	-	1 (100)	-
	Night blindness	-	2 (100)	3 (60)	2 (100)	1 (100)	1 (100)	2 (100)	1 (100)	-	2 (100)
BCVA Median (Range)	Right	3.0 (3.0)	0.0 (0.0)	1.0 (0.4 - 3.0)	1.8 (0.7 – 3.0)	0.2 (0.2)	0.5 (0.5)	0.6 (0.3 – 1.0)	0.4 (0.4)	3.0 (3.0)	0.5 (0.3 – 0.7)
	Left	3.0 (3.0)	0.0 (0.0)	0.8 (0.4 – 3.0)	1.8 (0.7 – 3.0)	0.1 (0.1)	0.8 (0.8)	0.6 (0.3 – 1.0)	0.4 (0.4)	0.3 (0.3)	0.5 (0.3 – 0.7)
Color, n(%)	Impaired			3 (60)	1 (50)		1 (100)	1 (50)	1 (100)		1 (50)
	Normal		2 (100.0)	2 (40)	1 (50)	1 (100)		1 (50)		1 (100)	1 (50)
	Cannot be assessed	1 (100)									
Lens, n(%)	Clear	1 (100)	1 (50)	1 (20)	-	1 (100)	-	1 (50)	1 (100)	-	1 (50)
	Nuclear sclerotic and posterior subcapsular cataract	-	-	-	-	-	-	-	-	1(100)	-
	Posterior subcapsular cataract	-	1 (50)	-	-	-	-	-	-	-	-
	Posterior subcapsular cataract, right; pseudophakic, left	-	-	-	-	-	-	-	-	-	1 (50)
	Pseudophakic	-	-	4 (80)	2 (100)	-	1 (100)	1 (50)	-	-	-
Fundus findings, n (%)	Bone spicule pigmentation in peripheral retina	1 (100)	2 (100)	5 (100)	2 (100)	1 (100)	1 (100)	2 (100)	1 (100)	1 (100)	2 (100)
	Arteriolar attenuation	1 (100)	2 (100)	5 (100)	1 (50)	1 (100)	1 (100)	2 (100)	-	1 (100)	2 (100)
	Disc pallor	-	-	2 (40)	-	-	1 (100)	-	-	-	-
	Peripapillary atrophy	-	-	1 (20)	-	-	-	-	-	-	-
Fundus autofluorescence findings, n(%)	Hyperfluorescent ring in parafovea	1 (100)	2 (100)	2 (40)	1 (50)	1 (100)	1 (100)	2 (100)	1 (100)		2 (100)
	Patchy hypo-fluorescence in retina	1 (100	2 (100)	3 (60)	2 (100)	1 (100)	1 (100)	2 (100)	1 (100)	1 (100)	2 (100)
	Location	Peripheral and central	Peripheral	Peripheral (1) Peripheral and central (2)	Peripheral	Peripheral	Peripheral and central	Peripheral and central; the rest of retina	Peripheral and central	Peripheral and central	Peripheral and central
	Confluent areas of hypo fluorescence in peripheral and central retina	-	-	2 (40)	-	-	-	-	-	-	-
Macular OCT findings, n(%)	Choroidal neovascularization	-	-	1 (20)	-	-	-	-	-	-	-
	Vitreomacular traction	-	-	-	-	-	-	-	-	-	1 (50.)
	Epiretinal membrane	-	-	2 (40)	-	1 (100)	-	-	-	-	-
	Hyperreflective foci in outer retinal layer	-	-	3 (60)	-	-	-	1 (50)	1 (100)	1 (100)	-
	Hyporeflective cystic spaces	-	-	1 (20)	-	-	-	1 (50)	-	-	-
	Loss of ellipsoid zone	-	2 (100)	4 (80)	2 (100)	1 (100)	1 (100)	2 (100)	1 (100)	1 (100)	2 (100)
	Zone	n/a	Peripheral	Peripheral and central	Peripheral (1) Peripheral and central (1)	Peripheral	Peripheral and central	Peripheral (1) Peripheral and central (1)	Peripheral and central	Peripheral and central	Peripheral (1) Peripheral and central (1)
Other associated manifestations		developmental delay; polydactyly; acquired nystagmus; hypogenitalism									hearing loss (1)

Table 2. Summary of genes found in the study

Family	Patient	Age	Sex	Genotype	Mutation	Heterozygosity	Inheritance	Classification
1	PH001	26	M	RPGR:c.2442_2445del:p.V814fs	Frameshift	N/A	X-linked recessive	Likely Pathogenic
2	PH002	44	F	FAM161A:c.1003C>T:p.R335X	Nonsense	Homozygous	AR	Pathogenic
	PH003	56	M	FAM161A:c.1003C>T:p.R335X	Nonsense	Homozygous	AR	Pathogenic
3	PH004	45	F	FLVCR1:c.1482C>A:p.N494K	Missense	Homozygous	AR	Likely Pathogenic
4	PH005	55	F	RGS7:c.679C>T:p.R227W	Missense	unknown	unknown	Under research
	PH006	56	F	RGS7:c.679C>T:p.R227W	Missense	unknown	unknown	
5	PH007	56	F	EYS c.2439C>A (p.Cys813*)	Nonsense	Heterozygous	AR	Pathogenic
6	PH008	34	F	EYS c.6416G>A(p.Cys2139Tyr) EYS c.8411dup(p.Thr2805Asnfs*7)	Missense Frameshift	Compound heterozygous	AR	Pathogenic Pathogenic
7	PH009	66	F	USH2A c.2802T>G (p.Cys934Trp) USH2A c.13969T>C (p.Trp4657Arg)	Missense Missense	Compound heterozygous	AR	Pathogenic VUS
8	PH010	34	M	USH2A, Exon 43, c.8605C>A (p.Pro2869Thr) USH2A, Exon 48, c.9527_9529del (p.Pro3176_Glu3177delinsGln)	Missense Frameshift	Compound heterozygous	AR	VUS VUS
9	PH011	45	M	CNGB1 c.2302A>C (p.Lys768Gln) CNGB1 c.2965G>A (p.Val989Met)	Missense Missense	Compound heterozygous	AR	VUS VUS
	PH012	37	M	CNGB1 , c.2302A>C (p.Lys768Gln) CNGB1 c.2965G>A (p.Val989Met)	Missense Missense	Compound heterozygous	AR	VUS VUS
10	PH013	17	M	BBS5 c.259-3C>G (Intronic) BBS5 c.259-3C>G (Intronic)	Missense Missense	Homozygous	AR	Likely Pathogenic Likely Pathogenic
11	PH014	46	F	EYS c.8545C>T (p.Arg2849*)	Nonsense	Heterozygous	AR	Pathogenic
12	PH015	59	M	EYS c.6416G>A (p.Cys2139Tyr) EYS c.3280del (p.Cys1094Valfs*69)	Missense Frameshift	Compound heterozygous	AR	Pathogenic Pathogenic
13	PH016	48	F	PDE6A c.1311T>A (p.Tyr437*) PDE6A c.1311T>A (p.Tyr437*)	Nonsense Nonsense	Homozygous	AR	Pathogenic Pathogenic
14	PH017	55	F	EYS, Deletion (Exon 13)	Frameshift/Nonsense	Heterozygous	AR	VUS
15	PH018	80	F	SPP2c.26C>T,(p.Thr9Met)	Missense	Heterozygous	AD	VUS

PH011 and PH012 belonging to 1 family presented with night blindness at a median age of 10.5 (range 10-11) and had a median BCVA of 0 in both eyes. They were found to have the same compound heterozygous missense variants in *CNGB1*, classified as VUS. Both had normal color vision. PH011 had clear lenses, while PH012 had posterior subcapsular cataracts. Both had bone-spicule pigmentation, patchy hypofluorescence in the peripheral retina, and peripheral loss of the ellipsoid zone on OCT.

PH002 and PH003 from 1 family presented with night blindness at a median age of 10 (range 7-13). The median BCVA on both eyes was 1.85 (range 0.7-3). They were found to have the same homozygous missense mutation at *FAM161A*, classified as pathogenic. PH002 had normal color vision, while PH003 had impaired color vision. Both presented with bone-spicule pigmentation in the retina and arteriolar attenuation. Both had patchy hypofluorescence in the peripheral retina, and only PH003 had a hyperfluorescent parafoveal ring. OCT showed loss of ellipsoid zone peripherally for PH002 and peripherally and centrally for PH003.

PH005 and PH006 from 1 family presented with night blindness at a median age of 35 (range 30-40). The median BCVA in both eyes was 0.6 (0.3-1.0). They were found to have the same missense mutation in *RGS7*, the heterozygosity and inheritance of which are still unknown. PH006 could read 24/24 Ishihara color plates, while PH005 could read none. Both presented with bone-spicule pigmentation and arteriolar attenuation in the retina. Both had patchy hypofluorescence in the peripheral retina, and PH005 had hypofluorescent parafoveal rings. OCT showed loss of peripheral ellipsoid zone

and cystoid macular edema for PH006, while PH005 had loss of ellipsoid zone peripherally and centrally **(Table 1).**

PH009 and PH010 from 2 families presented with night blindness at a median age of 31.5 (range 13-50). The median BCVA in both eyes was 0.5 (0.3-0.7). Both had compound heterozygous mutations. PH010 had normal color vision, while PH009 had impaired color vision. PH009 had posterior subcapsular cataract on her right eye. Both had bone-spicule pigmentation, arteriolar attenuation, patchy hypofluorescence in the retina, and hyperfluorescent parafoveal ring. OCT showed loss of peripheral ellipsoid zone in PH010, while PH009 had loss of central and peripheral ellipsoid zone and vitreomacular traction.

PH013 presented with blurred vision at age 2. The patient also presented with developmental delay, polydactyly, hypogenitalism, and acquired nystagmus at age 12. BCVA for both eyes was 3.0. Color testing with Ishihara plates was not assessed for this patient. Genotypic profile showed homozygous missense variant in BBS5 gene, classified as likely pathogenic. Fundus findings showed bone spicule pigmentation along the peripheral retina, with patchy hypofluorescence peripherally on fundus autofluorescence. OCT was not performed due to nystagmus **(Table 1).**

PH004 presented with night blindness at the age of 25. BCVA was 0.2 and 0.1 for the right and left eye respectively. Color vision was normal in both eyes. The patient had a homozygous missense variant of the FLVR gene, which was likely pathogenic. Fundus showed bone spicule pigmentation along the peripheral retina, arteriolar attenuation, and peripheral patchy hypofluorescence on fundus autofluorescence testing. OCT scan of the macula showed loss of ellipsoid zone and epiretinal membrane formation **(Table 1).**

PH016 initially presented with night blindness at the age of 8. BCVA was 0.5 and 0.8 for the right and left eye, respectively. Color testing was 1/24 for both eyes. The patient had a homozygous nonsense variant at the PDE6A gene, which was classified as pathogenic. Patient was pseudophakic in both eyes at the time of assessment. Fundus showed bone spicule pigmentation along the peripheral retina, arteriolar attenuation, and disc pallor. A central hyperfluorescent ring in parafovea with central and peripheral patchy hypofluorescence was seen on fundus autofluorescence. OCT scan of the macula showed loss of ellipsoid zone centrally and along the periphery **(Table 1).**

PH001 was noted to have night blindness at the age of 10. Color testing was impaired, with BCVA at 0.4 for both eyes. Genotypic profile of the patient showed a homozygous frameshift mutation at the RPGR gene that was noted to be likely pathogenic. Bone spicule formation was seen along the peripheral retina of both eyes. On fundus autofluorescence central hyperfluorescent ring in parafovea with patchy hypofluorescence centrally and along the periphery were seen. Macular OCT showed loss of ellipsoid zone and hyperreflective foci along the outer retinal layers **(Table 1).**

PH018 was noted to have blurring of vision at the age of 13. The patient had bilateral nuclear and posterior subcapsular cataracts with BCVA was 3.0 and 0.3 for the right and left eye, respectively. Patient had normal color vision. Genotypic profile of the patient showed a heterozygous missense mutation at the SPP2 gene, classified as a variant of unknown significance. Bone spicule formation was seen along the peripheral retina of both eyes with evidence of arteriolar attenuation. Fundus autofluorescence showed central hyperfluorescent ring in parafovea with patchy hypofluorescence centrally and along the periphery. Loss of ellipsoid zone was seen on OCT, with hyperreflective foci seen along the outer retinal layers.

DISCUSSION

In this study, 10 genes with 22 variants were identified. Among the 10 genes found in the study, 70% had an autosomal recessive inheritance, followed by X-linked recessive, autosomal dominant, and unknown inheritance at 10% each.

The *EYS* gene or eyes shut homolog gene is the largest gene in the human eye and is predominantly expressed in the photoreceptor cells of the retina.[5] There are no mammalian models on the *EYS* gene because it is not found in the genome of several

rodent species, but zebrafishes have been found to share a gene that is similar to the structure and sequence of the human *EYS* gene.[5,6] Zebrafishes lacking the function of the *EYS* gene were found to have disorganized retinal architecture and visual dysfunction.[6]

While it is less common in Western countries, EYS gene mutations were identified as the most common cause of nonsyndromic autosomal recessive RP in the East Asian region. In addition to Japan and Korea, it was found to be the most common gene involved in RP among the Thai population.[5,7,8] Other manifestations of *EYS* mutations are macular dystrophy and autosomal recessive cone-rod dystrophy.[5]

RP caused by mutations in the *EYS* gene present clinically as early night blindness and progressive visual field constriction, with visual acuity loss during the second decade of life. Affected patients usually retain their central vision until very late in life.[5] Most patients present with the classic hallmarks of RP, with bone spicule pigmentation, attenuation of the retinal vessels, and waxy pallor of the optic disc. OCT imaging shows relative preservation of the central retinal layers in the early stages of the disease, but progression toward the macular region is common in the later stages.[5,9]

Five patients presented with variants in *EYS*. Two patients have compound heterozygous mutations while three were heterozygous. Of the three patients with heterozygous variants, two were nonsense mutations, (*EYS*: c.2439C>A, c.8545C>T), and both have been previously reported in individuals with autosomal recessive RP.[10,11,12] The other heterozygous variant was a deletion of exon 13. This variant has not been reported in population database sets (gnomAD)[13] and individuals with *EYS* conditions. It is important to note that *EYS* – related RP is an autosomal recessive disease. These three individuals only have variants in one allele, despite having severe RP phenotypes. One possibility for these patients is that their other allele might be deep intronic.

The *RPGR or RP GTPase regulator* gene is located on the short arm of the X chromosome and encodes a protein that is essential for the viability of the outer segment of rod photoreceptors.[14,15] The gene has several isoforms, of which the isoform *RPGR*^{ORF15} is most strongly expressed in the retina. The exon ORF15 repetitive domain is a common site for mutations for X-linked RP.[15,16] In this study, PH001 has a frameshift mutation in exon ORF15 of the *RPGR* gene.

Mutations in the *RPGR* gene account for 70-90% of x-linked RP and up to 20% of all RP.[15] X-linked RP is associated with a severe phenotype that typically manifests in affected males within the first two decades of life. Initial symptoms usually present as night blindness and constriction of the visual fields, progressing to severe visual loss by the third or fourth decade.[15,16] Although X-linked RP is thought to only affect males, studies have shown that carrier females may also present with variable phenotypes, ranging from asymptomatic to severe retinal degeneration.[17]

A recent Phase I/II clinical trial evaluating the safety and efficacy of subretinal delivery of an adeno-associated viral vector (AAV) encoding codon-optimized human *RPGR* (AAV8.*co*RPGR) showed visual field improvements in 6 out of 18 patients included in the trial without significant safety issues.[18]

PH001 had a frameshift mutation in his *RPGR* gene (exon15:c.2442_2445del:p.V814fs), which is classified as a likely pathogenic variant. In the family pedigree, the PH001 has a male sibling affected with visual dysfunction, while no female relatives are affected, making it likely that the variant was inherited in an X-linked recessive manner. PH001's severe phenotype, manifesting as early loss of the ellipsoid zone centrally, is typical of *RPGR*-associated RP.

The *USH2A or usherin* gene encodes for usherin, a basement membrane protein expressed in the human fetal cochlea, eye, brain, and kidney.[16,17] Disruption of this gene in mice leads to progressive photoreceptor degeneration and nonprogressive hearing impairment. This suggests that usherin is required for the development of cochlear hair cells and the maintenance of retinal photoreceptors.[20]

Mutations in *USH2A* causes 10-15% of autosomal recessive nonsyndromic RP cases and 30-40% of Usher syndrome type 2 cases.[18] Usher syndrome type 2 patients present with congenital or

early unilateral or bilateral moderate-to-severe sensorineural deafness with normal vestibular function and RP that begins in the first or second decade of life, presenting with night blindness, concentric visual field loss, and visual acuity loss.[21,22,23]

Two patients from different families were found to have mutations in *USH2A* in this study. PH009 has compound heterozygous variants in her USH2A gene (c.2802T>G (p.Cys934Trp)) and (c.13969T>C (p.Trp4657Arg)), classified as pathogenic and VUS, respectively. As the second variant is still a VUS, it is possible that this patient has an undetected variant causing the disease. This patient presented with hearing loss in her right ear since childhood, along with classic signs of RP. PH010 has compound heterozygous mutations in his USH2A gene (c.8605C>A(p.Pro2869Thr)) and (c.9527_9529del (p.Pro3176_Glu3177delinsGln)), and presented with RP without hearing loss.

The *FAM161A* or family with sequence similarity, membrane A gene is expressed mainly in the retina, localized to the base of the photoreceptor connecting cilium, the ganglion cells, and synaptic regions of the outer and inner plexiform layers. [24, 25] Mouse studies suggest that deficiency in this gene causes retinal degeneration.[25] *FAM161A* mutations are the most frequent cause of autosomal recessive RP in the Israeli-Jewish population.[26] It has been identified in Dutch, Belgian, Palestinian, and Indian populations in much lower frequencies.[26,27,28]In a study on a mostly Jewish population, it was found that the most frequent initial symptom was night blindness, which began in childhood or adolescence. Patients with homozygous nonsense mutations tended to manifest with a lower visual acuity at younger ages. They also found that waxy pallor of the optic discs and attenuation of retinal vessels appear at relatively early ages, but bone spicule pigments appear later in life, with initial pigmentation often observed in the mid-periphery only after the age of 30.[26]

PH002 and PH003 are siblings that have the same homozygous nonsense mutation in their *FAM161A* gene (exon3:c.1003C>T:p.R335X), which are pathogenic variants. The older sibling, PH003, presented with more advanced disease compared to PH002.

FLVCR1 or feline leukemia virus subgroup C receptor 1 gene encodes for a transmembrane heme exporter protein that maintains the intracellular heme concentration.[29,30] In animal studies, it was found that FLVCR1 mRNA levels were most abundant in the retina, followed by the posterior column of the spinal cord, suggesting that mutations in *FLVCR1* causes retinal and posterior column degeneration via dysregulation of heme or iron homeostasis.[31] Mutations in *FLVCR1* can present with a wide variation, such as nonsyndromic RP, RP with mild cerebellar signs, and posterior column ataxia and RP.[32,33] Due to the low number of identified patients and variability in phenotype, genotype-phenotype correlations are still poorly-defined.[27] PH004 was found to have homozygous missense mutations in her *FLVCR1* (exon8:c.1482C>A:p.N494K) gene. She presents with typical RP, with relatively good vision, and has no signs of posterior column ataxia.

RGS7 or regulator of G protein signaling 7 gene is a member of the R7 subfamily of regulators of G protein signaling and is present at the dendritic tips of retinal depolarizing bipolar cells, along with *RGS6* and *RGS11*. [33] Animal studies have shown that electroretinogram results of true *RGS7*-null mice show prolonged b-wave implicit times at eye opening but this disappears at 2 months old.[33] In another study, the elimination of *RGS7* alone did not influence dark-adapted light-evoked responses.[34] In *RGS7* and *RGS11* double knock-out mice, the b-wave is no longer present. The mice also had a delayed onset and reduced magnitude of dark-adapted light-evoked responses.[33,34] Mutations in the *RGS7* gene have not been previously reported to cause RP, or any other pathology in humans. However, another protein in the same family caused by homozygous mutations in *RGS9* has been reported to cause RP (OMIM: 608415).[35]

In this study, 2 siblings, PH005 and PH006, had the same missense variant in the *RGS7* gene (exon10:c.679C>T:p.R227W). This variant has not been reported in any population database sets (gnomAD).[13] In-silico tools predicts this variant to be probably damaging as well (Polyphen).[36] However, despite these data, more studies are needed regarding this gene and its disease-causing mechanisms.

Cyclic nucleotide-gated (CNG) channels are non-selective cation channels expressed in

photoreceptors and olfactory neurons that translate light-mediated second messenger changes into voltage signals.[37,38] The CNG beta-1 or *CNGB1* subunit of the rod CNG channels is an important modulatory unit in phototransduction that is expressed exclusively in the retina, and mutations of which have been known to cause autosomal recessive RP, accounting for approximately 1 – 4% of cases.[39]

Typical profile of *CNGB1*-related mutations includes childhood-onset night blindness, peripheral visual field constriction in early adulthood, and preserved visual acuity through late adulthood. Phenotypic presentation includes fundus abnormalities typical of RP, with the characteristic bone-spicule pigmentation, varying degrees of macular atrophy, reduced midperipheral autofluorescence with central hyperautofluorescent ring, and a generally well-preserved macular morphology and central subfield thickness.[38] Recent reports have also documented cases of anosmia among patients with *CNGB1* mutations, accounting for the involvement of the *CNGB1b* isoform in olfactory transduction.[39,40.] Despite its wide spectrum of mutations, the characteristic slow progression of photoreceptor degeneration among affected patients provides a lengthy window of opportunity for therapeutic intervention via gene augmentation. Recent animal studies with AAV vectors have documented successful restoration of rod-driven light responses in *CNGB1* knockout mouse models of RP, showing promise in the application of gene augmentation therapy in the treatment of RP among humans.[37]

In this study, 2 siblings, PH011 and PH012, were found to have the same VUS in *CNGB1* (c.2302A>C (p.Lys768Gln)) and (c.2965G>A (p.Val989Met)). Both variants have not been reported in population database sets (gnomAD) nor in individuals with *CNGB1*-related conditions.[13] In-silico tools also predict both variants to be highly disruptive to protein structure and function (SIFT, PolyPhen-2, Align-GVGD).[36,41,42,43] Lastly, the presentation of RP and segregation of the variants in the siblings make these variants to be likely causative to their RP. The RP in these siblings are typical of *CNGB1*-related RP, with good central visual acuity and relatively well-preserved ellipsoid zone centrally. *BBS5* or Bardet-Biedl Syndrome 5 gene is one of the 24 identified genes proven to be implicated in Bardet-Biedl Syndrome (BBS), a rare, autosomal recessive ciliopathy presenting with early-onset progressive RP, postaxial polydactyly, obesity, renal malformations, learning disability, and male hypogenitalism.[44] Included as one of the components of the BBSome complex, *BBS5* in conjunction with *BBS8* are complex proteins required for ciliary trafficking that ultimately contribute to the regulation of the primary cilium seen in photoreceptor outer segments. Mutations of this protein usually account for 2% of BBS cases with no particular ethnic specificity.[44,45]

Young *et al.*reported that patients affected by *BBS5* mutations present with advanced retinal degeneration with severe visual impairment, obesity, male hypogenitalism, brachydactyly and/or syndactyly but without polydactyly.[46] Fundus imaging shows presence of macular dystrophy with evidence of outer retinal structure loss and typical central hypofluorescence with surrounding hyperfluorescence. [47]

PH013 was found to have homozygous intronic missense mutations in his *BBS5* gene (BBS5 c.259-3C>G). Apart from the typical presentation of RP, PH013 also presented with developmental delay, hypogenitalism, and polydactyly. This presentation is typical of BBS, making the mutation likely pathogenic.

Phosphodiesterase 6A or *PDE6A* gene is a rod-specific effector enzyme that encodes the alpha subunit of cyclic guanosine monophosphate (cGMP) phosphodiesterase which is central to rod phototransduction.[48,49] It is primarily involved in the processes of transmission and amplification of visual signal and pathologic variants generally result with cGMP dysregulation and defective biochemical signaling of light stimuli. Incidence of *PDE6A* mutations among patient with RP occurs in 1-4% of autosomal recessive RP cases, accounting for approximately 36,000 of cases worldwide.[49] The disease manifests with a highly symmetrical rod-cone dystrophy, with primary loss of rod photoreceptors followed by secondary cone photoreceptor loss, leading to mild to moderate visual impairment.[49,50] Typical RP imaging findings are also seen, with pigmentary deposits seen on fundus examination, thinning of outer retinal layers involving the retinal periphery and macular center, and evidence of

ellipsoid zone disruption.[51,52] A few subset of patients also present with cystoid macular edema, epiretinal membranes, and macular holes, occurring in decreasing frequency.[53]

Pre-clinical trials done on mice showed promise with gene augmentation therapy, resulting in effective photoreceptor cell rescue when delivered early in the course of the disease.[53] Present studies have been geared towards the use of AAV vectors in human gene therapy trials among patients with inherited retinal dystrophies, including those with *PDE6A*-associated RP.[52]

PH016 was found to have a homozygous missense mutation in her *PDE6A* gene c.1311T>A (p.Tyr437*), which is already classified as a pathogenic variant. Her phenotype is highly typical of RP.

One of the more recently discovered and relatively poorly characterized genes implicated in RP is the *SPP2* gene. It encodes for phosphoprotein 24 (spp-24) located in multiple tissues including the liver, kidneys, plasma, and retina, and is known to form a tertiary structure similar to cystatin. Abnormalities in its cellular processing result in alteration in the function and activities of cathepsins, which in the retina leads to retinal pigment epithelium dysfunction and photoreceptor degeneration.[54]

SPP2 gene mutations result in autosomal dominant RP, and affected patients present with typical bilateral RP fundus findings, including bone-spicule pigmentation, waxy disc pallor, and macula-preserving retinal atrophy. Onset of symptoms typically starts during early adulthood and vision appears to be relatively well-preserved.[54]

PH018 was found to have a heterozygous missense variant in her *SPP2* gene c.26C>T,(p.Thr9Met), which is classified as a VUS. Given her clinical presentation, however, it is highly likely that this variant is responsible for causing her phenotype.

One limitation of this study was the absence of formal visual field tests and electroretinograms which could more completely assess visual and retinal functions. Given the genetic heterogeneity of RP and the lack of studies in the Filipino population, we recommend that large-scale studies on the genetic etiologies of RP be done. Genetic testing of the parents of the probands with VUS may also be helpful in determining pathogenicity, as well as determining prognosis and recurrence risk of the disease. Finally, the success of clinical trials for gene therapy highlights the need for more genetic studies to be done in the Filipino population, so that affected patients may be identified and be given the chance to participate in future trials.

In summary, this study identified 10 causative genes of RP from 15 families using next-generation sequencing. One possible novel gene, *RGS7*, was identified in 1 family, and novel variants of *CNGB1* was also identified in another family. The study noted that in those with nonsense EYS mutations had more severe phenotypes.

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