UNIVERSIDADE D COIMBRA FACULDADE DE MEDICINA

MESTRADO INTEGRADO EM MEDICINA – TRABALHO FINAL

MIGUEL ÂNGELO APÓSTOLO PIMENTEL

GENOMIC PROFILE AND GENOTYPE-PHENOTYPE CORRELATIONS IN PATIENTS WITH NON-SYNDROMIC RETINITIS PIGMENTOSA IN A TERTIARY CARE CENTRE IN PORTUGAL

ARTIGO CIENTÍFICO

ÁREA CIENTÍFICA DE OFTALMOLOGIA

Trabalho realizado sob a orientação de:

DOUTOR JOÃO PEREIRA FIGUEIRA

DR. JOÃO PEDRO MARQUES

ABRIL/2020

GENOMIC PROFILE AND GENOTYPE-PHENOTYPE CORRELATIONS IN PATIENTS WITH NON-SYNDROMIC RETINITIS PIGMENTOSA IN A TERTIARY CARE CENTRE IN PORTUGAL

Miguel Ângelo Apóstolo Pimentel[1]

João Pedro Marques[1,2]

João Pereira Figueira[1,2]

[1] Faculty of Medicine, University of Coimbra (FMUC), Coimbra, Portugal

[2] Department of Ophthalmology, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal

E-mail: miguel.aa.pimentel@gmail.com

Institutional Address: Polo III – Polo das Ciências da Saúde

Azinhaga de Santa Comba, Celas, 3000-548 Coimbra

APRIL/2020

INDEX

RESUMO

Introdução: A retinopatia pigmentar (RP) é a distrofia hereditária da retina mais prevalente e uma das principais causas irreversíveis de comprometimento visual e cegueira em todo o mundo. Em Portugal, o perfil genético dos doentes com RP não sindrómica é ainda desconhecido. Este estudo tem como objetivos caracterizar a etiologia genética de uma série de casos de RP não sindrómica, bem como estabelecer eventuais correlações genótipo-fenótipo.

Métodos: Estudo unicêntrico, transversal, que incluiu 50 doentes consecutivos (de 39 famílias) com o diagnóstico clínico de RP não sindrómica, seguidos num centro hospitalar terciário em Portugal. Todos os doentes realizaram estudo molecular, seja através de sequenciação Sanger, painéis de sequenciação de nova geração ou sequenciação do exoma completo.

Resultados: Encontrámos variantes causadoras de doença (clinicamente relevantes) em 25/39 famílias (64.10%), totalizando 22 variantes causadoras de doença em 10 genes diferentes. Os genes *EYS, IMPG2, RPGR* e *RHO* foram os mais frequentemente implicados, explicando 72% dos casos resolvidos. Na RP com hereditariedade autossómica recessiva, o gene *EYS* foi o mais frequente, enquanto que os genes *RPGR* e *RHO* foram os mais frequentes na RP ligada ao cromossoma X e na RP autossómica dominante, respetivamente.

Conclusão: Este estudo preliminar é o primeiro estudo a reportar o perfil genético de doentes portugueses com RP não sindrómica. A obtenção de dados robustos baseados em estudos populacionais é o primeiro passo para um melhor aconselhamento genético e prognóstico, bem como para uma melhor orientação para futuras intervenções terapêuticas.

Palavras chave: Retinopatia Pigmentar, Distrofias hereditárias da retina, Distrofia de bastonetes-cones, Correlação genótipo-fenótipo, Sequenciação de nova geração

ABSTRACT

Introduction: Retinitis Pigmentosa (RP) is the most frequent inherited retinal dystrophy and a major cause of visual impairment and blindness worldwide. In Portugal, the genomic profile of patients with non-syndromic RP has never been reported. This study aimed to characterize the genomic landscape of Portuguese patients with non-syndromic RP and to establish possible genotype-phenotype correlations.

Methods: Cross-sectional, single-centre study conducted in 50 consecutive patients (from 39 families) with a clinical diagnosis of non-syndromic-RP, followed at a tertiary care hospital in Portugal. Patients underwent genetic testing through Sanger sequencing of a suspected gene, next generation sequencing panels, whole exome sequencing (WES)-based next generation sequencing panels or WES.

Results: We found disease-causing mutations in 25/39 patients (64.10%), totalizing 22 pathogenic variants identified in 10 genes. *EYS, IMPG2, RPGR* and *RHO* were the most frequently implicated genes, explaining 72% of the solved cases. Within autosomal recessive cases, *EYS* was the most frequently identified gene, while *RPGR* and *RHO* were the most common among X-linked and autosomal dominant cases, respectively.

Conclusion: This preliminary study is the first study to characterize the genomic profile of non-syndromic RP in Portugal. Achieving strong population-based data is the first step towards better genetic and prognostic counselling as well as guidance for future therapeutic interventions.

Key words: Retinitis Pigmentosa, Inherited retinal dystrophy, Rod-cone dystrophy, Genotype-phenotype correlation, Next generation sequencing

INTRODUCTION

Retinitis Pigmentosa (RP) is the most frequent inherited retinal dystrophy (IRD),[1] with an estimated prevalence of 1:4000.[2] The disease may be either syndromic (20-30%) or non-syndromic and is a major cause of visual impairment and blindness worldwide.

The age of onset is variable but legal blindness at working age is common, posing substantial socioeconomic implications and leading to a significant decrease in the patient's quality of life.[1, 3]

The first visual symptoms are night blindness and visual field constriction due to the degeneration and death of rod photoreceptors, followed by decreased daylight and central vision, due to secondary cone involvement.[1, 4] Other symptoms may include reading difficulties, photophobia, dyschromatopsia and photopsia.[5]

Despite its progressive character, RP has a high phenotypic variability, mostly because of its genetic heterogeneity. In fact, non-syndromic RP is associated with mutations in approximately 90 genes.[6] Additionally, different mutations in the same gene or even the same mutation in different family members can cause distinctive phenotypes while mutations in different genes can cause similar phenotypes, leading to complex genotype-phenotype correlations.[5]

RP follows a mendelian inheritance pattern – autosomal dominant (AD), autosomal recessive (AR) or X-linked (XL) – or, rarely, a digenic trait.[7] Nevertheless, the majority of cases occur sporadically (simplex cases).[1] Next Generation Sequencing (NGS) enables the simultaneous screening of multiple genes and nowadays is a cost effective method to characterize causative variants in heterogeneous diseases.[1, 8] Using NGS panels, a molecular diagnosis of RP is now possible in up to 70% cases. Establishing a molecular diagnosis in IRD patients provides support for the clinical diagnosis, allows genetic counselling and prenatal testing and identifies patients who would benefit from novel gene-based therapies.[5, 9, 10] However, genetic profiles vary dramatically among regions and ethnic groups, highlighting the importance of obtaining reference population-based data.[9]

In Portugal, the genomic profile of patients with non-syndromic RP has never been reported. By using the database of a Portuguese IRD referral hospital, this study aims to 1) evaluate the number of cases of non-syndromic RP in which the causative mutation could be identified; 2) describe the most frequent genes associated with non-syndromic RP and 3) establish genotype-phenotype correlations using multimodal retinal imaging.

METHODOLOGY

Cross-sectional, single-centre study including 50 consecutive patients (from 39 families) with a clinical diagnosis of non-syndromic-RP, followed at the Retinal Dystrophies Clinic of Centro Hospitalar e Universitário de Coimbra (CHUC). Only patients with genetic testing results available until January 2020 were included in the study. Clinical diagnosis was based on patient and family history along with typical changes on dilated fundus examination such as optic disc pallor, patches of outer retinal atrophy, bone spicule-like pigment clumping and attenuation of retinal vessels (Figure 1). Additionally, electrophysiology, visual field testing and multimodal retinal imaging – colour fundus photography (CFP), spectral domain optical coherence tomography (SD-OCT), OCT angiography (OCTA) and fundus autofluorescence (FAF) - were used to complement the clinical examination.

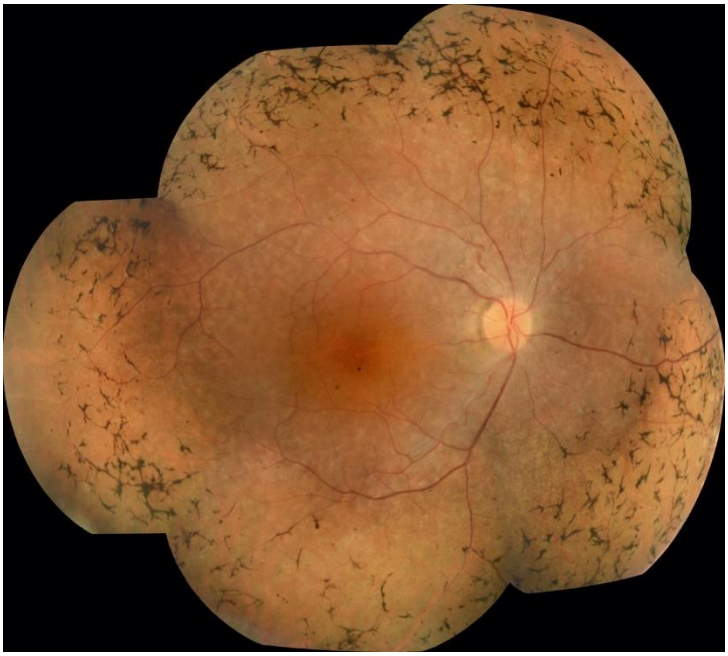

Figure 1 Typical fundus findings of retinitis pigmentosa. This colour fundus photography depicts the right eye of a 23-year-old patient with autosomal-recessive non-syndromic NR2E3-related retinitis pigmentosa. Patches of outer retinal atrophy are seen around the vascular arcades while bone spicule-like pigment clumping is observed in the mid-periphery. Mild attenuation of retinal vessels is present.

From the family history, patients were classified as AR-RP, AD-RP, XL-RP or unknown (when family history was negative or unknown).

Peripheral blood samples were collected according to the manufacturer's specifications for whole-blood DNA extraction. Genetic testing was clinically-oriented in all probands and included Sanger sequencing of a suspected gene, whole exome sequencing (WES)-based NGS panel (187 genes - Table 1) or WES. The option for Sanger sequencing as a first line

genetic testing was chosen for cases in which the phenotype was highly suggestive of a specific gene (eg. Sector Retinitis Pigmentosa and *RHO* gene – Figure 6). Whenever clinically appropriate, multiplex ligation-dependent probe amplification (MLPA) was also used. In all cases of suspected XL inheritance unsolved by NGS, the mutational hot spot in RPGR exon ORF15 was analysed. Whenever possible, segregation analysis was performed in family members. The identified variants were classified according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines.

All patients had access to genetic counselling from a dedicated medical genetics specialist and were granted the possibility of issuing the "Rare Disease Card", which includes relevant information such as the name and the orphacode of the disease, the name and contact of the Reference Centre, along with specific health recommendations to be provided in case of an emergency.

The study adhered to the tenets of the Declaration of Helsinki. All patients signed an informed consent for genetic testing and another allowing the introduction of their data on the retina.pt data base, approved by the local Ethics committee.

Table 1 - WES-based NGS panels of 187 genes that were tested in our cohort
ABCA4, ABHD12, ACO2, ADAM9, ADAMTS18, ADGRV1, AGBL5, AHI1, AIPL1, ALMS1, ARHGEF18, ARL2BP, ARL6, ATF6, ATOH7, BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, BEST1, C1QTNF5, C21orf2, C2orf71, C8orf37, CA4, CABP4, CACNA1F, CAPN5, CC2D2A, CDH23, CDH3, CDHR1, CEP164, CEP290, CEP78, CERKL, CHM, CIB2, CLN3, CLRN1, CNGA1, CNGA3, CNGB1, CNGB3, CNNM4, COL4A1, CRB1, CRX, CSPP1, CTNNB1, CYP2R1, CYP4V2, DHDDS, DHX38, EFEMP1, ELOVL4, ERCC6, ERCC8, EYS, FAM161A, FLVCR1, FSCN2, FZD4, GNAT1, GNAT2, GNPTG, GPR143, GPR179, GRK1, GRM6, GUCA1A, GUCA1B, GUCY2D, HARS, HGSNAT, HK1, HMX1, IDH3B, IFT140, IMPDH1, IMPG1, IMPG2, INPP5E, IQCB1, KCNJ13, KCNV2, KIAA1549, KIF11, KLHL7, LCA5, LRAT, LRIT3, LRP5, LZTFL1, MAK, MERTK, MFRP, MKKS, MKS1, MYO7A, NDP, NEK2, NMNAT1, NPHP1, NPHP3, NPHP4, NR2E3, NRL, NYX, OAT, OFD1, OTX2, PANK2, PCDH15, PCYT1A, PDE6A, PDE6B, PDE6C, PDE6G, PEX1, PEX2, PEX7, PHYH, PLA2G5, PRCD, PROM1, PRPF3, PRPF31, PRPF4, PRPF6, PRPF8, PRPH2, PRPS1, RAB28, RAX2, RBP3, RBP4, RD3, RDH11, RDH12, RDH5, RGR, RGS9, RHO, RLBP1, ROM1, RP1, RP1L1, RP2, RP9, RPE65, RPGR, RPGRIP1, RPGRIP1L, RS1, SAG, SCAPER, SDCCAG8, SEMA4A, SLC24A1, SLC38A8, SNRNP200, SPATA7, TIMP3, TMEM237, TOPORS, TRIM32, TRPM1, TSPAN12, TTC8, TUB, TULP1, USH1C, USH1G, USH2A, VCAN, VPS13B, WDPCP, WDR19, WHRN, ZNF408, ZNF423, ZNF513

Other data

A retrospective analysis of every included patient's file was conducted and the following data was collected: gender, date of birth, naturality, age of onset of symptoms, date of diagnosis, follow up time, general findings on the ophthalmological examinations and ancillary tests, including CFP, FAF, SD-OCT, visual field testing and electrophysiology. Family history and, whenever positive, inheritance pattern and presence of inbreeding in the family were also collected.

Statistical analysis

The study population demographics, clinical and imaging characteristics were summarized using traditional descriptive methods. Regarding the relative frequency of the different variants and inheritance patterns, the analysis was performed by affected families (n=39). Cases were considered solved if a pathogenic or likely pathogenic variant was found and unsolved if no pathogenic variants were found or in cases where the pathogenicity of variants of unknown significance (VUS) could not be confirmed. Thus, the diagnostic yield was calculated from the number of variants classified as pathogenic or likely pathogenic.

Graphical representations were built on Microsoft Excel Office 365.

RESULTS

Cohort characteristics

We included 50 patients from 39 pedigrees. The ages of onset were comprised between 7 and 67 years old, with a mean patients' age of 35.31 ± 15.32 years old. Age of onset organized in subgroups along with baseline demographic data can be found on Table 2I. All patients were Portuguese, from 12 of the 18 Portugal Continental Districts and 1 patient from The Azores Islands.

Table 2 – Cohort Clinical Characteristics

	Patients, n (%)		
	Solved Cases	**Unsolved Cases**	**TOTAL**
Sex			
Male	18 (58.06%)	10 (52.63%)	28 (56.00%)
Female	13 (41.94%)	9 (47.37%)	22 (44.00%)
Age of onset			
<10 years	0 (0.00%)	2 (10.53%)	2 (4.00%)
10-60 years	27 (87.10%)	16 (84.21%)	43 (86.00%)
>60 years	2 (6.45%)	1 (5.26%)	3 (6.00%)
Unknown	2 (6.45%)		2 (4.00%)
Consanguinity			
Yes	6 (19.36%)	6 (31.58%)	12 (24.00%)
No	21 (67.74%)	11(57.89%)	32 (64.00%)
Unknown	4 (12.90%)	2 (10.53%)	6 (12.00%)
Family History			
Yes	20 (64.52%)	11 (57.89%)	31 (62.00%)
No	10 (32.26%)	7 (36.85%)	17 (34.00%)
Unknown	1 (3.22%)	1 (5.26%)	2 (4.00%)
TOTAL	31 (62.00%)	19 (38.00%)	50 (100.00%)

Diagnostic yield and genetic findings

Disease-causing genotypes were found in 25/39 families (64.10%), thus considered genetically solved (Table 3). A total of 22 disease-causing mutations were identified in 10 different genes (Table 4 and Figure 2). Among the genetically unsolved cases, in 6 families (42.86%) no clinically significant variants were found, while in 8 families (57.14%) we found VUS. Since further investigation to determine the pathogenicity of these VUS could not be performed or was not completed at the time of writing this manuscript, these cases were also considered unsolved.

Table 3 - Distribution and Detection Rate in 39 different families with non-syndromic RP

	Families, n (%)				
Inheritance pattern	***AR***	***AD***	***X-Linked***	***Unknown***	**TOTAL**
Solved Cases	9 (60.00%)	3 (75.00%)	4 (100.00%)	9 (56.25%)	25 (64.10%)
Unsolved Cases	6 (40.00%)	1 (25.00%)	0 (0.00%)	7 (43.75%)	14 (35.90%)
TOTAL	15 (38.50%)	4 (13.33%)	4 (13.33%)	16 (53.33%)	39 (100.00%)

The most frequently implicated genes were *EYS, RPGR, IMPG2* and *RHO*, explaining 72% of all solved cases (Figure 2).

All cases where the inheritance pattern could not be established clinically (n=9) revealed to be AR, with 9 different variants identified in 6 different genes (Figure 3). Overall, AR-RP was the most commonly observed inheritance pattern (61.54%; 24/39), with mutations in *EYS* explaining 44% of solved cases (18/24). The remaining cases were caused by *IMPG2, RPE65, PROM1, NR2E3, PCARE, BBS2* and *USH2A* (Figure 4). In AD-RP (10.26%; 4/39), all solved cases (3/4) were *RHO*-related, while in X-Linked RP (10.26%; 4/39), pathogenic variants in *RPGR* (including the ORF15 region) explained all cases.

No specific clinical characteristics such as later age of onset, atypical fundus features, presence of phenocopies or consanguinity could be used to separate solved from unsolved case.

Table 4 – Mutated genes and Pathogenic variants

Gene	Reference Sequence	Mutation	Amino acid change	Zygosity	Pathogenicity	Families (n)
EYS	NM_001142800.1	c.5928-2A>G p			Pathogenic	1
		c.4120C>T c.8003G>T	p.(Arg1374*) p.(Cys2668Phe)	CH	Pathogenic	1
		c.8779T>C	p.Cys2927Arg	HM	Pathogenic	1
		Chr6(GRCh37):g.65707475-?_65767620+?del; NM_001142800.1:c.2024-?_c.2259+?del – Premature STO P codon	(p.(?)		Pathogenic	1
		c.4120C>T	p.(Arg1374*)	HM	Pathogenic	1
		c.225del c.(2023+1_2024-1)_(2259+1_2260-1)del	p.(Cys742Leufs*36)	CH	Pathogenic	1
		c.5928-2A>G c.4120C>T	(p.Arg1374*)	CH	Pathogenic	1
		c.8834G>A Deleção on exons 13 and 14	p(Gly2945Glu)	CH	Pathogenic	1
IMPG2	NM_016247.3	c.634delT	p.(Ser212Glnfs*19)	HM	Pathogenic	1
		NA	NA		Probably Pathogenic	1
		NA	NA		Probably Pathogenic	1
BBS2	NM_031885.3	c.627_628del c.401C>G	p.(Cys210Serfs*20) p.(Pro134Arg)	CH	Pathogenic	1
NR2E3	NM_014249	c.119-2A>C	p.?	HM	Pathogenic	1
PCARE	NM_001029883.3	c.3099delinsCCAGG	p.(Val1034Glnfs*74)	HM	Pathogenic	1
PROM1	NM_006017.2	c.869del	p.(Ser290Ilefs*2)	HM	Pathogenic	1
RPE65	NM_000329.2	c.1022T>C	p.Leu341Ser	HM	Pathogenic	2
USH2A	NM_206933.2	c.907C>A	p.(Arg303Ser)	HM	Pathogenic	1
RHO	NM_000539.3	c.316G>A	p.(Gly106Arg)	HT	Pathogenic	2
		c.403C>T	(p-Arg135Trp)	HT	Pathogenic	1
RPGR	NM_000328.2	c.2615_2616delAG	p.(Glu872Glyfs*206	HeM	Pathogenic	1
		c.1243_1244del	p.(Arg415Glyfs*37)	HeM	Pathogenic	2
		c.1261dup	p.(Ser421Phefs32)	HeM	Pathogenic	1

Legend: NA: Not Available; HM: Homozygosity; HT: Heterozygosity; CH: Compound Heterozygosity; HeM: Hemizygosity

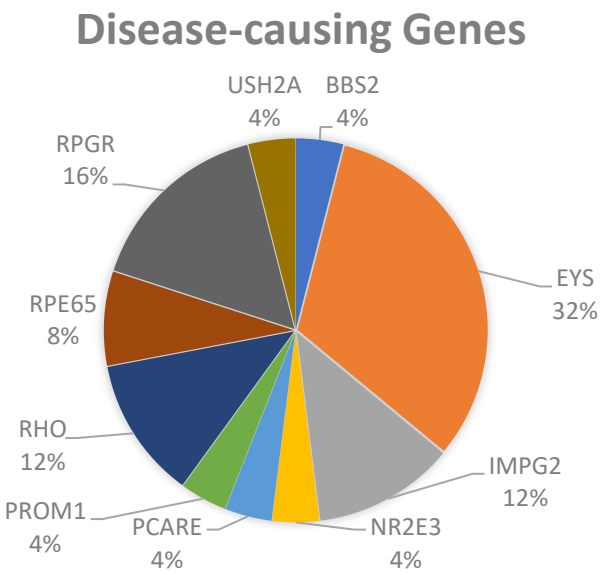

Figure 2 *Proportion of disease-causing genes in the 25 different families with genetically-solved RP.*

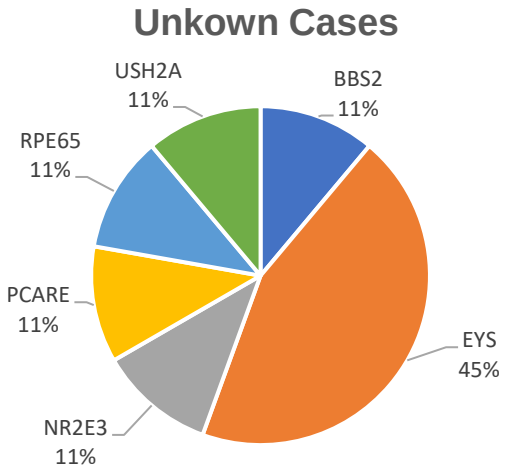

Figure 3 *Proportion of mutated genes in patients where the inheritance pattern could not be established clinically. All of these cases proved to be AR.*

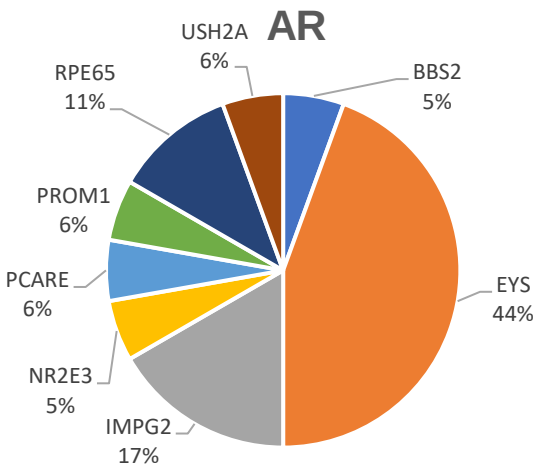

Figure 4 *Proportion of mutated genes in families with AR-RP (cases where a clear inheritance pattern could not be established clinically but proved to be AR are included)*

Genotype phenotype correlation

As far as best corrected visual acuity (BCVA) is concerned, and considering all patients (n=50), overall mean baseline BCVA was 66.13 ETDRS letters and mean final BCVA was 58.00 letters, with a mean follow up time of 403.34 days. In the most frequently mutated genes (*EYS*, IMPG2, *RPGR* and *RHO)* baseline mean BCVA was 76.72, 58.90, 72.00 and 55.50 letters and final mean BCVA was 70.36, 48.90, 58.88 and 55.50, respectively. The distribution of final BCVA according to the involved gene can be seen in Figure 5.

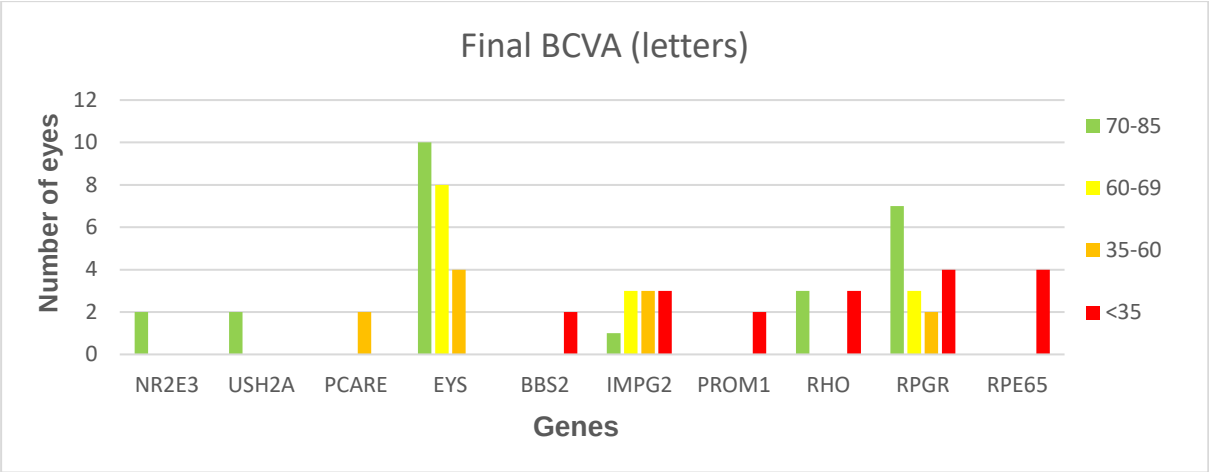

Figure 5 *Distribution of final best-corrected visual acuity (BCVA) in ETDRS letters according to the affected gene.*

Another important clinical feature was the previous or actual diagnosis of cataract, which is known to accompany the RP phenotype early in the disease course. History of previous or present cataract was found in 63,21% of the evaluated eyes (n=55) and was classified as posterior subcapsular in all cases. Phacoemulsification had already been performed in 22 eyes (40%).

Sector retinitis pigmentosa, a distinctive and rare RP phenotype, was observed in 1 patient (Figure 6). Although establishing genotype-phenotype correlations in RP is very difficult, some distinctive phenotypes, like sector RP, are highly suggestive of a particular gene. In this case, the positive family history (mother with sector RP) and the patient's phenotype on multimodal retinal imaging were consistent with RHO-related RP, further confirmed by Sanger sequencing.

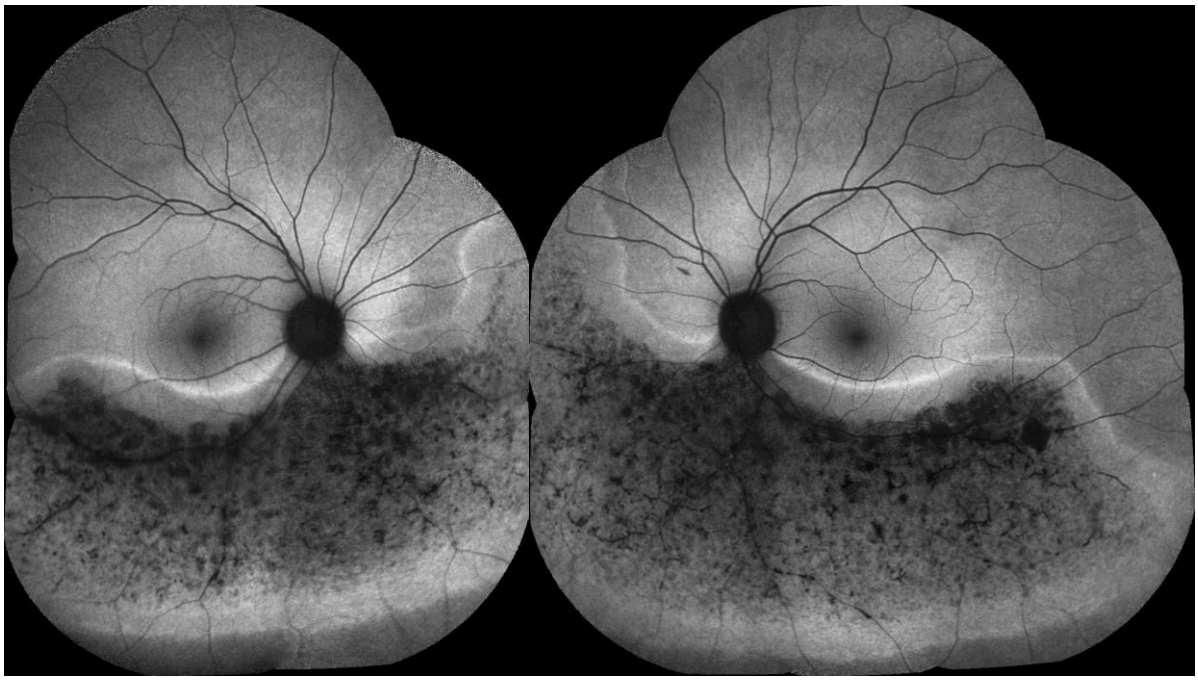

Figure 6 *Fundus Autofluorescence of a patient with sector RP associated with mutations in the RHO gene.*

DISCUSSION

This study is the largest ever reported cohort of Portuguese patients with non-syndromic RP and the first to describe the genomic landscape of the disease in our country. The overall diagnostic yield was 64.10% (25/39), which is a little bit lower than some of the most recent investigations in China (72.08%; 896/1243)[9] and Germany (70%; 81/116)[3]. However, it was much higher than a Japanese study (29.6%; 356/1204)[4]. These differences may eventually be justified by our small sample size and because segregation analysis was not always possible in patients with VUS.

There is no standard between relative frequency of the inheritance patterns in the literature, which is explained by mutational specificities of the populations. Nevertheless, AR mutations are the most frequent, followed by AD and XL mutations. In this study, AR-RP was the most frequent inheritance pattern and XL-RP was slightly more prevalent than AD-RP. However, these are just preliminary results from the IRD-PT Study, a nationwide study aiming to characterize the genomic landscape of IRDs in the Portuguese population.

Pathogenic variants in 4 genes (*EYS*, *IMPG2, RHO* and *RPGR*) explained almost 3/4 of solved cases. These findings are in consonance with findings in other populations, namely in Japan[4], China[9] and Germany[3] but not with the findings in the Ashkenazi Jewish population, where the most frequently mutated genes are *MAK* and *DHDDS*[3, 4, 9, 11]. This corroborates the general idea that population-specific frequent variants influence the difference in the proportion of causative genes across populations.

In this study, 14 cases (35,90%) remained genetically unsolved. Cases with no clinically significant variants found (n=6) may be due to causative variants in non-coding regions of these genes or in genes not yet known to underlie retinal degeneration, or less likely due to mutations in regions below optimal coverage. Interestingly, when consanguinity was present (24%; 12/50 patients), the causative mutations could only be found in 50%. On the other hand, in cases with a positive family history, a molecular diagnosis was achieved in 64.52%, thus confirming the assumed inheritance.

A recent study by Birtel et al[12] reported that patients with a later onset of symptoms (>30 years old), atypical RP phenotypes or phenocopies and absent family history would be more likely to be unsolved. However, in our cohort, no common features were shared by the unsolved cases. This is probably because of the considerably smaller sample size.

This study has several limitations. First, the number of families is relatively small as we only included those with genetic testing results available until January 2020. However, these are only preliminary results and inclusion will continue as part of the IRD-PT Study. The limited number of included patients prevented strong genotype-phenotype correlations so only descriptive findings were reported. Finally, segregation analysis was not possible or

incomplete in a significant number of cases where VUS were found, thus affecting the percentage of solved cases.

CONCLUSION

Achieving strong population-based data is the first step towards better genetic and prognostic counselling as well as guidance for future therapeutic interventions. Despite the limited number of included patients, this study demonstrates the genetic heterogeneity of non-syndromic RP in Portugal and a highly satisfactory detection rate of disease-causing mutations using clinically-oriented genetic testing. Our preliminary results shed light on the genomic profile of Portuguese patients coming from 2/3 of the countries districts, thus guaranteeing a great geographical representation.

REFERENCES

1. Martin-Merida, I.; Avila-Fernandez, A.; Del Pozo-Valero, M.; Blanco-Kelly, F.; Zurita, O.; Perez-Carro, R.; Aguilera-Garcia, D.; Riveiro-Alvarez, R.; Arteche, A.; Trujillo-Tiebas, M. J.; Tahsin-Swafiri, S.; Rodriguez-Pinilla, E.; Lorda-Sanchez, I.; Garcia-Sandoval, B.; Corton, M.; Ayuso, C., Genomic Landscape of Sporadic Retinitis Pigmentosa: Findings from 877 Spanish Cases. *Ophthalmology* 2019.
2. Hamel, C., Retinitis pigmentosa. *Orphanet J Rare Dis* 2006, *1*, 40.
3. Birtel, J.; Gliem, M.; Mangold, E.; Muller, P. L.; Holz, F. G.; Neuhaus, C.; Lenzner, S.; Zahnleiter, D.; Betz, C.; Eisenberger, T.; Bolz, H. J.; Charbel Issa, P., Next-generation sequencing identifies unexpected genotype-phenotype correlations in patients with retinitis pigmentosa. *PLoS One* 2018, *13* (12), e0207958.
4. Koyanagi, Y.; Akiyama, M.; Nishiguchi, K. M.; Momozawa, Y.; Kamatani, Y.; Takata, S.; Inai, C.; Iwasaki, Y.; Kumano, M.; Murakami, Y.; Omodaka, K.; Abe, T.; Komori, S.; Gao, D.; Hirakata, T.; Kurata, K.; Hosono, K.; Ueno, S.; Hotta, Y.; Murakami, A.; Terasaki, H.; Wada, Y.; Nakazawa, T.; Ishibashi, T.; Ikeda, Y.; Kubo, M.; Sonoda, K. H., Genetic characteristics of retinitis pigmentosa in 1204 Japanese patients. *J Med Genet* 2019.
5. Birtel, J.; Eisenberger, T.; Gliem, M.; Muller, P. L.; Herrmann, P.; Betz, C.; Zahnleiter, D.; Neuhaus, C.; Lenzner, S.; Holz, F. G.; Mangold, E.; Bolz, H. J.; Charbel Issa, P., Clinical and genetic characteristics of 251 consecutive patients with macular and cone/cone-rod dystrophy. *Sci Rep* 2018, *8* (1), 4824.
6. Motta, F. L.; Martin, R. P.; Filippelli-Silva, R.; Salles, M. V.; Sallum, J. M. F., Relative frequency of inherited retinal dystrophies in Brazil. *Sci Rep* 2018, *8* (1), 15939.
7. Hartong, D. T.; Berson, E. L.; Dryja, T. P., Retinitis pigmentosa. *Lancet* 2006, *368* (9549), 1795-809.
8. Huang, X. F.; Huang, F.; Wu, K. C.; Wu, J.; Chen, J.; Pang, C. P.; Lu, F.; Qu, J.; Jin, Z. B., Genotype-phenotype correlation and mutation spectrum in a large cohort of patients with inherited retinal dystrophy revealed by next-generation sequencing. *Genet Med* 2015, *17* (4), 271-8.
9. Gao, F. J.; Li, J. K.; Chen, H.; Hu, F. Y.; Zhang, S. H.; Qi, Y. H.; Xu, P.; Wang, D. D.; Wang, L. S.; Chang, Q.; Zhang, Y. J.; Liu, W.; Li, W.; Wang, M.; Chen, F.; Xu, G. Z.; Wu, J. H., Genetic and Clinical Findings in a Large Cohort of Chinese Patients with Suspected Retinitis Pigmentosa. *Ophthalmology* 2019, *126* (11), 1549-1556.

10. Holtan, J. P.; Selmer, K. K.; Heimdal, K. R.; Bragadottir, R., Inherited retinal disease in Norway - a characterization of current clinical and genetic knowledge. *Acta Ophthalmol* 2019.

11. Kimchi, A.; Khateb, S.; Wen, R.; Guan, Z.; Obolensky, A.; Beryozkin, A.; Kurtzman, S.; Blumenfeld, A.; Pras, E.; Jacobson, S. G.; Ben-Yosef, T.; Newman, H.; Sharon, D.; Banin, E., Nonsyndromic Retinitis Pigmentosa in the Ashkenazi Jewish Population: Genetic and Clinical Aspects. *Ophthalmology* 2018, *125* (5), 725-734.

12. Birtel, J.; Gliem, M.; Oishi, A.; Muller, P. L.; Herrmann, P.; Holz, F. G.; Mangold, E.; Knapp, M.; Bolz, H. J.; Charbel Issa, P., Genetic testing in patients with retinitis pigmentosa: Features of unsolved cases. *Clin Exp Ophthalmol* 2019, *47* (6), 779-786.