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CLINICAL ASSESSMENT OF ROD-CONE DYSTROPHY PATIENTS CARRYING RHODOPSIN MUTATIONS

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LIST OF ABBREVIATIONS

adRP – Autosomal dominant Retinitis Pigmentosa

arRP – Autosomal recessive Retinitis Pigmentosa

CSNB – Congenital Stationary Night Blindness

ERG – Electroretinography

GPCR – G protein-coupled receptor

IOL – Intraocular lens

OCT – Optical Coherence Tomography

OD – Right eye

OS – Left eye

RCD – Rod-Cone Dystrophy

RHO – Rhodopsin gene

ROS – Rod outer segment

RPE – Retinal pigment epithelium

XLRP – X-linked Retinitis Pigmentosa

ABSTRACT

Rod-cone dystrophies (RCD) are a heterogeneous group of genetic retinal disorders characterized by the progressive loss of rod and cone photoreceptors, leading in most cases to severe visual impairment. It is one of the most common inherited diseases of the retina with a unique set of clinical characteristics that make it a complex disease associated to distinct inheritance patterns.

Mutations in the rhodopsin gene (*RHO*) are suggested to be the most common cause of autosomal dominant retinitis pigmentosa (adRP); nevertheless, the prevalence of *RHO* mutations in the Portuguese population has not been established. In this study, direct cycle sequencing was used to analyze all five coding exons and adjacent intronic regions of the *RHO* gene in 48 Portuguese probands with different forms of non X-linked RP (XLRP).

Two novel *RHO* missense mutations were identified in 2 of the 48 unrelated tested probands; the c.180 T>C transition (exon 1) leading to a p.Y60H substitution, identified in patient AAV, is located at the cytoplasmic end of the first transmembrane domain, whereas the c.207 C>T transition (exon1) leading to a p.R69C substitution, identified in patient MBC, is located in the first intra-cytoplasmic loop. Both mutation replace important amino acid residues that interfere with protein folding (class II mutations).

The mutation frequency of this Portuguese sample is 4,16% (2/48) which is not concordant with earlier studies in other Caucasian populations. This is probably due to the geographic isolation for many centuries and high consanguineous rates in our population.

Complete clinical assessment disclosed typical autosomal dominant cases but with early-onset of symptoms, which might be related to the position and function of the

amino acid replaced in the protein. Differences related to rhythms of progression of the disease could be explained by differences in the genetic background or environmental factors.

RESUMO

A distrofia de bastonetes e cones é um grupo heterogéneo de doenças genéticas da retina que são caracterizadas pela perda progressiva dos fotoreceptores, geralmente provocando graves perturbações da visão. É uma das doenças hereditárias da retina mais frequentes com um conjunto único de características clínicas que a tornam uma doença complexa associada a diferentes padrões de hereditariedade. As mutações no gene da rhodopsina (*RHO*) são, provavelmente, a causa mais frequente de Retinopatia Pigmentada autossómica dominante, contudo, a prevalência na população Portuguesa não está estabelecida.

Neste estudo, utilizamos a sequenciação directa para analizar os cinco exões e regiões intrónicas adjacentes do gene da rodopsina (*RHO*) em 48 probandos portugueses com diferentes formas de RP não ligadas ao cromossoma X.

Identificámos duas novas mutações *missense* no gene da rodopsina em 2 dos 48 probandos testados; a transição c.180 T>C (exão 1) que provoca uma substituição p.Y60H, identificada no paciente AAV, está localizada na extremidade citoplasmática do primeiro domínio transmembranar; enquanto, a transição c.207 C>T (exão1) que provoca uma substituição p.R69C, identificada no paciente MBC, está localizada na primeira *loop* intra-citoplasmática. Ambas as mutações provocam a substituição de aminoácidos importantes que interferem com a estrutura terciária da proteína (mutações de classe II).

A prevalência de mutações na amostra de Portugueses é 4,16% (2/48), sendo inferior a estudos anteriores em populações caucasianas. Isto, provavelmente, é devido ao isolamento geográfico durante vários séculos e a uma alta taxa de consanguinidade na nossa população.

A avaliação clínica completa revelou casos típicos de retinopatia pigmentada autossómica dominante, contudo com um início precoce dos sintomas, o que pode estar ligado à posição e função dos aminoácidos substituídos na proteína. As diferenças relacionadas com o ritmo de progressão da doença podem ser explicadas por diferenças no *background* e factores ambientais.

KEY WORDS

Autosomal dominant RP (adRP), Genotype/phenotype correlation, Missense mutations, Photoreceptors degeneration, Rhodopsin gene (RHO), Rod-cone dystrophies (RCD), Portuguese population

INTRODUCTION

Rhodopsin is a G protein-coupled receptor (GPCR) - family A [1], with a seven α-helical transmembrane architecture, that is covalently bound via a protonated Schiff base to the light sensitive chromophore 11-cis-retinal, which is the only light-sensitive protein in the visual transduction cascade. [2-3]

The importance of rhodopsin arises from its primary role in vision (initiation of phototransduction cascade). It constitutes up to 85% of the total amount of protein in the rod outer segment (ROS) [4] and is present both in the plasma membrane and in the lamellar sides of the disks.

Using somatic cell hybrid studies, Nathans and Hogness assigned the human Rhodopsin gene (*RHO*, MIM #180380), which consists of five exons, to 3q21-ter. [5] Mutations in the *RHO* were first described in 1990. [6-7]

Although there are reports of autosomal recessive Retinitis Pigmentosa (arRP) [8-11], congenital stationary night blindness (CSNB) [12-15] and retinitis punctata albescens [16], almost all mutations in the *RHO* cause autosomal dominant RP (adRP). [17] In the adRP, the second most frequent mode of inheritance of RP (15% to 20%), 20-25% of families have mutations in rhodopsin. [17]

So far, over 120 mutations have been found in the *RHO* gene in association with RP. [17] They are located in all three domains of rhodopsin, namely the intradiscal, the transmembrane and the cytoplasmic domains.

Soon after the identification of mutations in *RHO*, additional studies with transgenic mice indicated that defective folding (class II) [18-19] and transport (class I) [20-21] of rhodopsin to the membrane are the primary defects in adRP. [22-26]

RP belongs to the group of pigmentary retinopathies, a generic name that covers all retinal dystrophies presented with a loss of photoreceptors and retinal pigment

deposits. [27] The word ''retinitis'' is a misnomer because retinal inflammation does not play a prominent role in the disease's pathophysiology [28] and the word "pigmentosa" refers to an associated discoloration of the retina, which is detectable on eye examination.

RP is the leading cause of inherited retinal degeneration - associated blindness worldwide [28-33] with a prevalence approximately 1 in 3,000 to 1 in 5,000 individuals [28, 30-33], affecting approximately 1.5 million people. [32, 34-35]

The most common form of RP is a rod-cone dystrophy (RCD), characterized by the primary degeneration of rods followed by a secondary loss of cone sensitivity in the later stages. Patients typically present a history of night blindness followed by a mid-peripheral visual field loss. In the later stages of the disease, cone degeneration becomes more evident with the loss of central vision acuity and color vision defects. RP is usually non-syndromic but there are also many syndromic forms, the most frequent being Usher syndrome. [36-37]

Degeneration of photoreceptors associated with RP, although stimulated by various processes, is primarily genetically programmed. [28-34] Despite reports of families where the RP phenotype follows a non-mendelian inheritance pattern [38-42] the vast majority are inherited as mendelian traits. Most cases are monogenic, but the disease is nevertheless very heterogeneous genetically; and most genes involved in the disease are linked to only one form of inheritance (exceptions, mutation *NRL, RP1* and, exceptionally, *RHO*). [43]

AdRP are usually the mildest forms (slowest progression), with some cases starting after the age of 50 [44], however severe disease can also appear. [45] Most pedigrees show complete penetrance, and yet, adRP can vary greatly from individual to individual even within the same pedigree. [44]

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Objective measures of photoreceptor sensitivity, such as electroretinogram, are much more reliable than symptoms for diagnosis of RP and grading its severity.

In this study, we propose to identify prevalence of RHO mutations in Portuguese patients with non-X linked forms of RP. Then, perform a complete clinical assessment including novel techniques for better structural and functional assessment of retinal degeneration with special care given to the study of rod and cone photoreceptors. Finally, identify potential genotype/phenotype correlation of patients with different mutations on the *RHO* gene.

POPULATION AND METHODS

Patients with RP/RCD were collected from our Center of Excellence for Hereditary Eye Diseases from de Department of Ophthalmology, University Hospital of Coimbra, between 1995 and 2010. A total of 48 probands with adRP, arRP, unknown patterns of inheritance, and cases without a family history (isolated) were collected during this period.

Detailed phenotypic characterization was performed, including family history, geographic provenance, best-corrected visual acuity (BCVA), slit-lamp examination, fundus examination using a non-contact 78 D lens. Fundus images were acquired in accordance to the International accepted guidelines using a Zeiss fundus camera with VISUPAC^TM Digital Imaging System (Carl Zeiss, Meditec, Jena, Germany) and a Pan-Retinal camera (OptomapR) (Optos plc, Dunfermline, Scotland, UK). Visual fields were assessed using a Humphrey Visual Field Analyzer i-Series (Carl Zeiss Ophthalmic Systems Inc, Dunblin, CA, USA), in accordance with the manufacturer's guidelines. Ganzfeld electroretinography (ERG) was performed in accordance with the ISCEV (International Society for Clinical Electrophysiology of Vision) guidelines. Clinical

assessment was completed with fundus autofluorescence imaging and optical coherence tomography (OCT) (HRAII and Spectralis OCT, respectively; Heidelberg Engineering, Dossenheim, Germany).

Peripheral blood samples with EDTA anticoagulant were collected from each patient. Genomic DNA was extracted using an Automated Extractor (BioRobots EZI, Qiagen, Hilden, Germany). The exons of the RHO gene, including the intron-exon boundaries, were PCR-amplified with previously described primers. [46]Sequencing reactions were performed using the 4-dye terminator cycle sequencing ready reaction kit (Big Dye DNA sequencing kit, Applied Biosystems, Foster City, CA, USA). Sequence products were resolved in a ABI Prism 3130 (Applied Biosystems).

This study was approved by the local ethics committee and followed the tenets of the Declaration of Helsinki. Informed consent was obtained from the participating individuals or their guardians prior to the collection of clinical data and genomic samples.

RESULTS

We screened the major gene for adRP, the *RHO* gene, for underlying rod-cone dystrophy by direct sequencing of the coding exons and flanking intronic regions in each proband. Two novel *RHO* missense mutations were identified, representing a c.180 T>C transition (exon 1) leading to a p.Y60H substitution (Fig. 1.1) and a c.207 C>T transition (exon1) leading to a p.R69C substitution (Fig. 1.2) in 2 of the 48 unrelated tested probands. Thus, the overall allele mutation frequency of this Portuguese sample is 4,16% (2/48) (Table I). Both mutations affect highly conserved amino acid residues and are not present in the healthy control population.
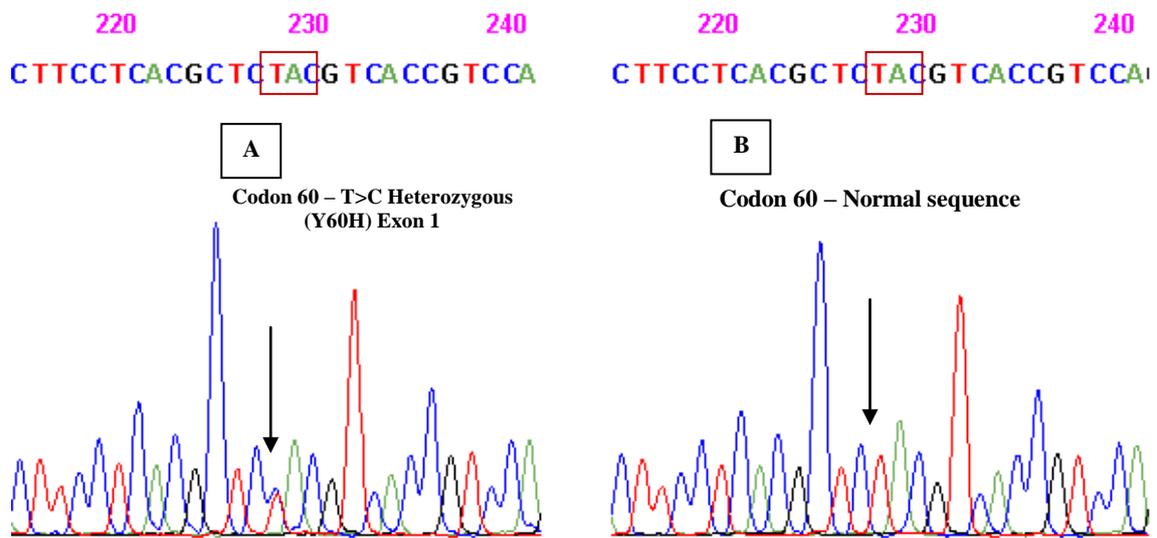
Figure 1.1 - Direct sequencing of the coding region of exon 1, patient AAV. **A-** Heterozygous missense mutation (TAC60CAC); **B-** Normal sequence around codon 60 of exon 1.
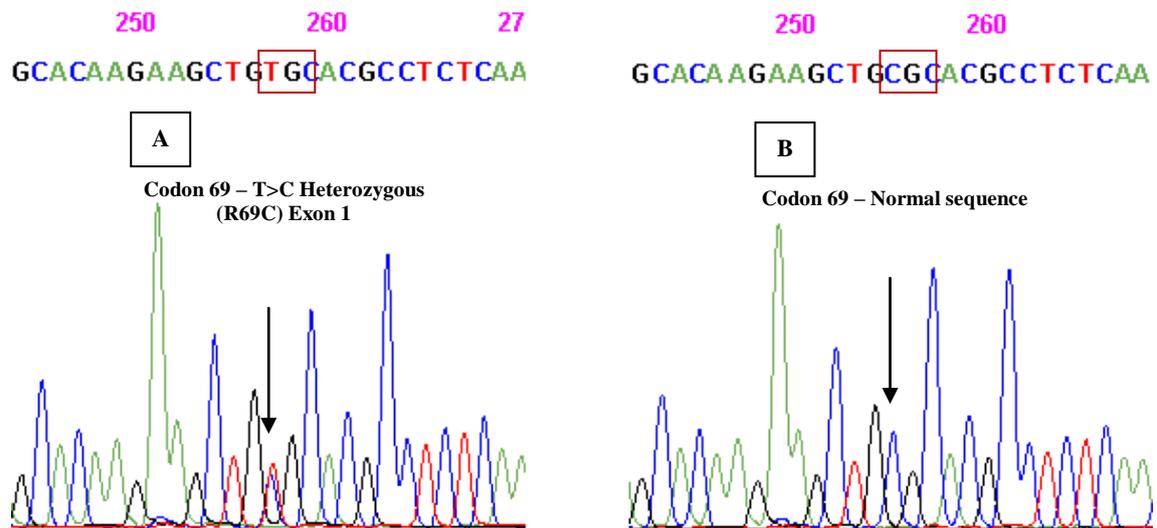

Figure 1.2- Direct sequencing of the coding region of exon 1, patient MBC. 1. **A-** Heterozygous missense mutation (CGC69TGC); **B-** Normal sequence around codon 69 of exon 1.

Table I- Sequence variation detected in *RHO* of patients.

Variation	Effect	Prediction	Frequency in patients	Note
c.180 T>C	p.Y60H	Damaging	1/48	Novel
c.207 C>T	p.R69C	Damaging	1/48	Novel

Both probands with heterozygous *RHO* mutations have clinical symptoms and signs of RP. They were available for clinical investigation, and the examination results are summarized in Table II.

AAV is a 57 year-old single male, born to non-consanguineous parents and no past family history of retinopathies (Fig. 2). His first disease symptoms started around age 7 with complaints of night blindness. Changes in the visual field were first noted during adolescence, with slow constriction of visual fields in parallel with progressive loss of vision. Photophobia became a problem after the fourth decade of life. At present his best corrected visual acuity is light perception with good projection for both eyes.

Ophthalmic examination disclosed abolished pupillary reflexes, absence of nystagmus, normal ocular motility with orthotropia for near and distance. Slit-lamp examination revealed bilateral pseudophakia with posterior chamber intra-ocular lens (IOL) and transparent posterior capsules. Dilated fundus examination (Fig. 3) depicted a pale optic disc, extremely narrow retinal vessels, scattered atrophy of the retinal pigment epithelium (RPE) with macular involvement and bone spicules distributed in the mid- and far periphery. This clinical picture is symmetrical in both eyes.

Complete phenotypical characterization included spectral-domain OCT (Fig. 4) that revealed significant disorganization of the RPE/photoreceptor interface, granular deposits in the outer retina and thinning of the neurosensory retina. Autofluorescence imaging (Fig. 5) demonstrated a macular hyperfluorescent ring surrounded by globular

areas of complete RPE atrophy, very thin retinal vessels and relative hypofluorescence in the perifoveal area. Ganzfleld ERG was completely flat. We did not perform multifocal ERG in this patient. Very limited visual acuity did not allow the use of Humphrey Visual field analysis.
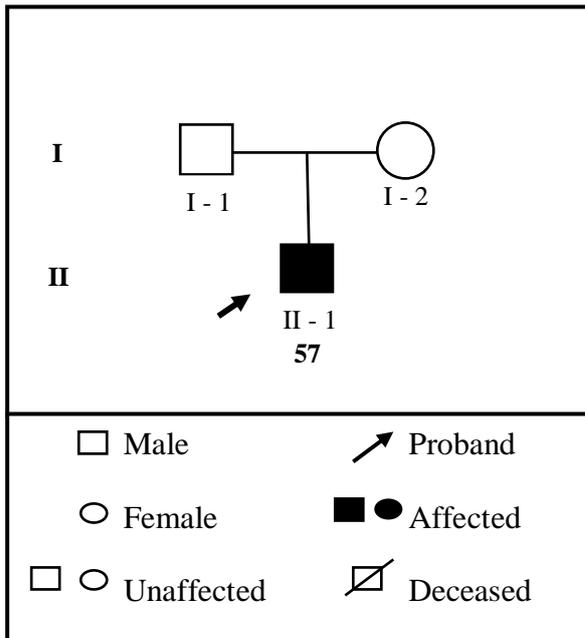

Figure 2- Pedigree of patient AAV who carries a c.180T>C transition leading to a p.Y60H substitution (simplex RP case). Patients II-1(AAV) is heterozygous for the mutation; unaffected family members are I-1 and I-2.
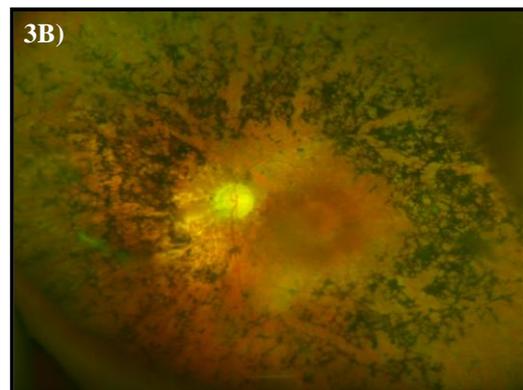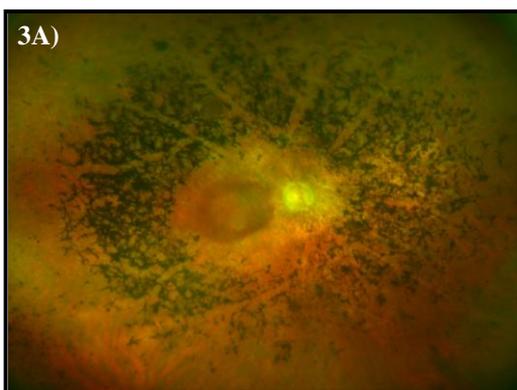

Figure 3- OptomapR images showing 200° fundus pictures. 3A (Right eye) Significant optic pallor, thin vessels, densely pigmented mid-periphery and scattered pigment bone spicules in the far periphery. Relative preservation of the inferior-nasal mid-periphery (less pigmented) is observed. 3B (Left eye) a symmetrical picture is observed for the contralateral fundus.
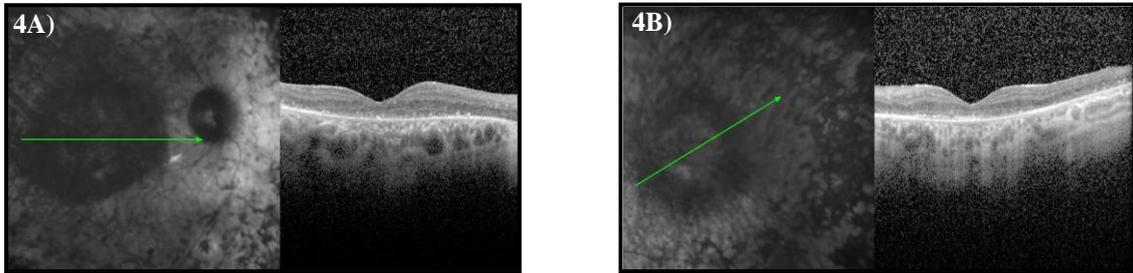
Figure 4- Spectral-domain OCT images line passing through the central macular area. Relative preservation of retinal thickness in the perifovea contrast with significant peripheral atrophy (macula). Disorganization of outer retinal layers. Inset 4A right eye; inset 4B left eye.
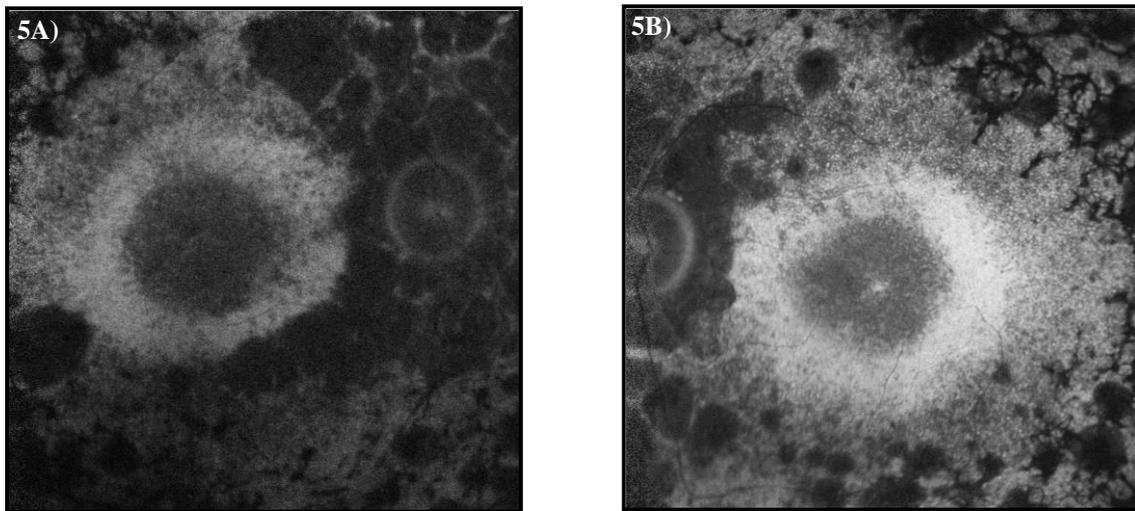

Figure 5- Auto-fluorescence images. 5A: right eye; 5B: Left eye. Macular hyperfluorescent ring surrounded by globular areas of complete RPE atrophy, very thin retinal vessels and relative hypofluorescence in the perifoveal area. Left eye displays a thicker and more hyperfluorescent ring.

MBC is a 59 year-old married male, born to non-consanguineous parents. Family history showed that his two elder brothers also had RP, but the youngest was unaffected; by questionnaire, his parents do not seem to be affected and have distinct geographical origin (Fig. 6). Symptoms included early-onset night vision disturbances (night blindness before age 10), constricted visual fields by confrontation and asymmetric decreased vision starting at the third decade of life. Photophobia became a problem after the fifth decade of life representing a mild to moderate impairment. At present his best corrected visual acuity is OD (right eye): 6:10 and OS (left eye): 3:10.

Complete ophthalmic assessment disclosed reduced pupillary reflexes, absence of nystagmus, normal ocular motility with orthotropia for near and distance. Slit-lamp examination revealed bilateral pseudophakia with posterior chamber IOL and transparent posterior capsules. Dilated fundus examination (Fig. 7 and 8) reveals patchy and asymmetrical areas of chorioretinal atrophy, optic disc pallor, narrow retinal blood vessels and bone spicule deposits distributed in the mid- and far periphery. This clinical picture is symmetrical in both eyes.

Further phenotypical characterization included spectral-domain OCT (Fig. 9) that revealed significant disorganization of the outer and inner layers of the neurosensory retina, a thick posterior hyaloid membrane/fibroglial proliferation that eliminates the typical central foveal pit and granular deposits in the outer retina. The loss of the outer retinal structures in atrophic areas is associated with increased deep backscatter and subretinal pseudocystic lesions that seem to correspond to vascular structures. Autofluorescence imaging (Fig. 10) revealed areas of granular RPE clumps with hyperautofluorescence interspersed with globular atrophic areas of RPE and outer retina. No typical hyperfluorescent ring was detected in this patient. Ganzfleld ERG was

completely flat. We did not perform multifocal ERG in this patient. Humphrey Visual field analysis demonstrated constricted visual field, less than 5° centrally (Fig.11).
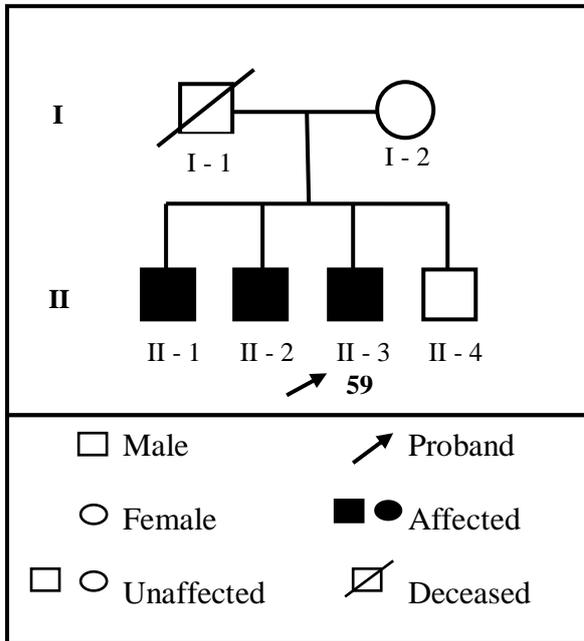

Figure 6- Pedigree of patient who carries a c.207 C>T transition leading to a p.R69C substitution in patient with presumed autosomal dominant RP. Patients II-1 (MBC) is heterozygous for the mutation; II-1 and II-2 are affected and unaffected family members are I-1, I-2 and II-4.
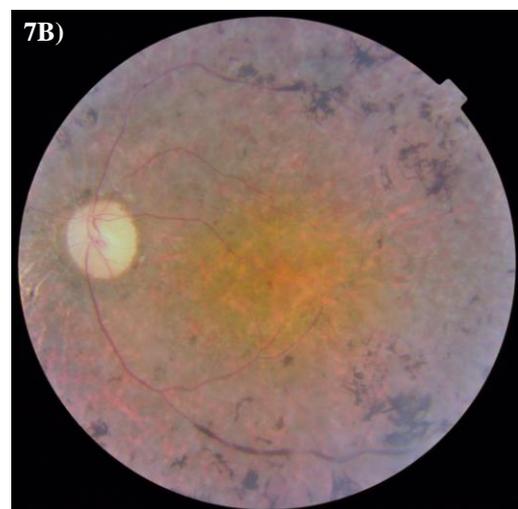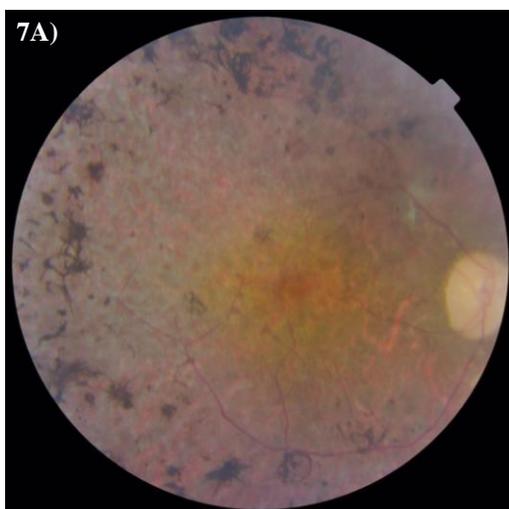
Figure 7- Fundus photography: 7A: Right eye: optic atrophy, significantly thin retinal vessels, patch atrophy of RPE; beaten bronze macula; peripheral pigmented bone spicules. 7B Left eye: same aspect; noteworthy the fact that there is paravascular pigment clumping along the inferior temporal arcade and also the superior temporal arcade.
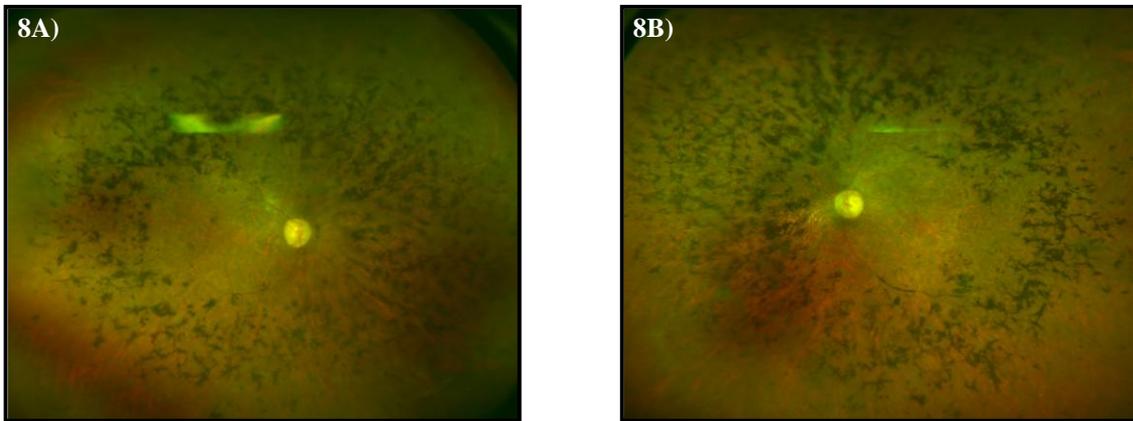
**Figure 8-** OptomapR images showing 200º fundus pictures. 8A (Right eye) Optic atrophy, thin vessels, pigmented mid-periphery and scattered pigment bone spicules in the far periphery. 8B (Left eye) a symmetrical picture is observed for the contralateral fundus.
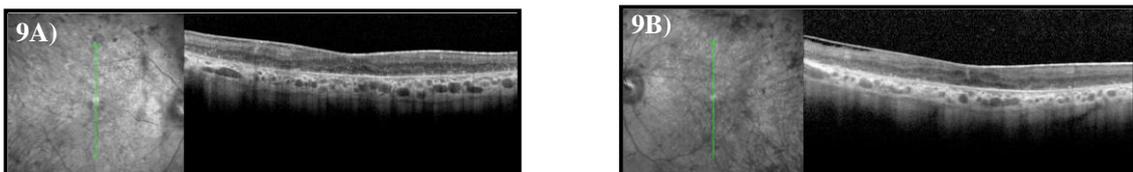
Figure 9- Spectral domain OCT. 9A (Right eye) and 9B (Left eye) revealed significant disorganization of the outer and inner layers of the retina, a thick posterior hyaloid membrane/fibroglial membrane, no central foveal pit and granular deposits in the outer retina. Sub-RPE increased deep backscatter and subretinal pseudocystic lesions correspond to choroidal vascular structures.
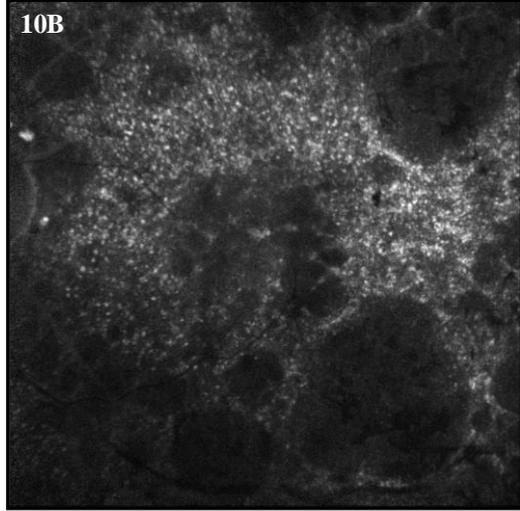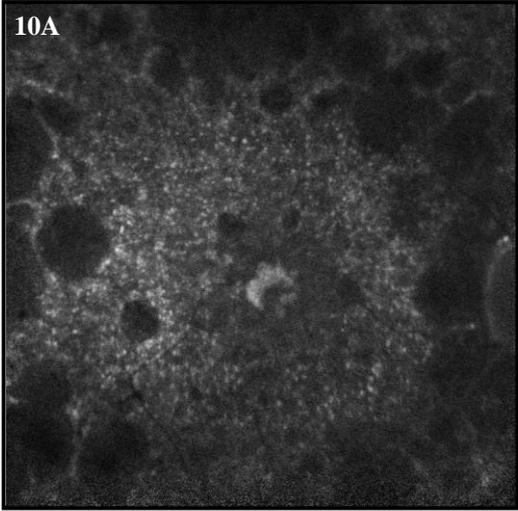
Figure 10- Auto-fluorescence images. Right (10A) and left (10B) eyes: areas of granular hyperfluorescent RPE clumps surrounded by globular atrophic areas of RPE and outer retina.
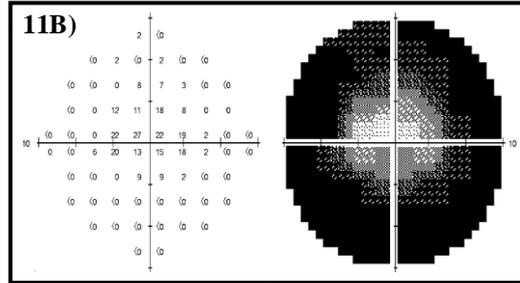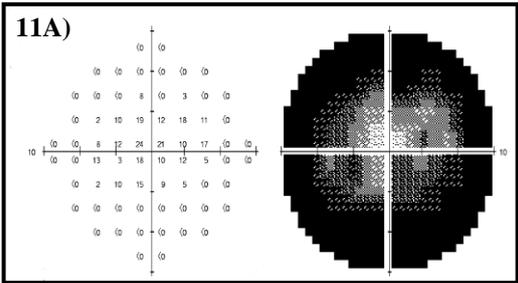
Figure 11- Humphrey visual field (10/2). 11A: Right eye; 11B: Left eye: constricted visual field, less than 5° centrally (Fig.11).

Table II- Clinical information from individuals with *RHO* variations.

ID	Variations	Gender	Age (yrs)	Age at onset	Inheritance	First symptom	BCVA	Fundus changes	ERG responses (Ganzfeld)		Humphrey Visual Field Analysis
									Rod	Cone	
AAV	c.180 T>C	M	57	7	Simplex case	Night Blindness	OD: LP with good projection OS: LP with good projection	RP; optic nerve atrophy, narrow vessels; RPE atrophic changes	Flat	Flat	Not recordable
MBC	c.207 C>T	M	59	50	Presumed AD	Night Blindness	OD: 6/10 OS: 3/10	RP; optic nerve atrophy, narrow vessels; RPE atrophic changes	Flat	Flat	Constricted VF, less than 5° centrally

Abbreviations: M – Male; AD – Autosomal dominant; LP- Light perception; VF – Visual Field

DISCUSSION

The present study provides a useful clue regarding the frequency of *RHO* mutations in the Portuguese population. The genetic screening reported here has identified two novel *RHO* missense mutations in 2 of the 48 unrelated tested probands. The c.180 T>C transition (exon 1) leading to a p.Y60H substitution, identified in patient AAV, is located at the cytoplasmic end of the first transmembrane domain, whereas the c.207 C>T transition (exon1) leading to a p.R69C substitution, identified in patient MBC, is located in the first intra-cytoplasmic loop (Fig. 12).
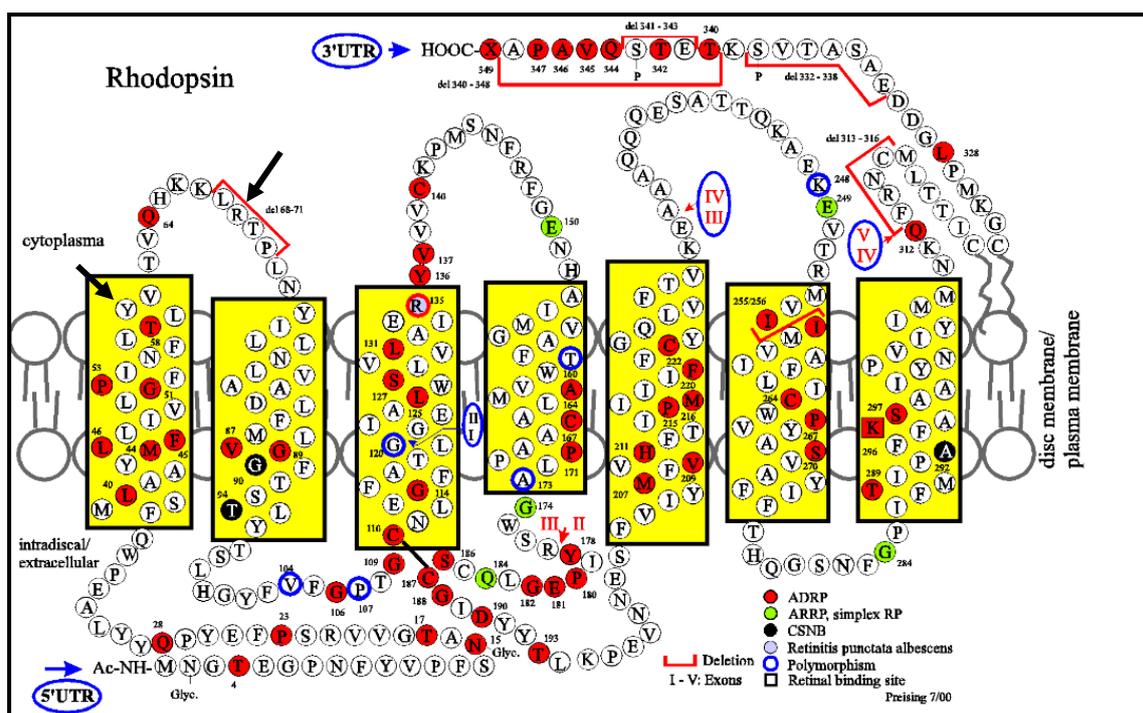

Figure 12- Schematic Model of human rhodopsin showing the locations of mutations described in rhodopsin, reported to date. Two novel mutations reported here are identified with arrows (adapted from Preising 07.2000).

Most of the rhodopsin mutations identified to date in subjects with adRP have been found in only one or just a few families. [6-7, 22, 47-50]

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Although *RHO* is the first gene implicated [6-7] and probably the most studied gene in RP, the great number of rare mutations suggests that many additional mutations in the rhodopsin gene remain to be discovered.

The finding of 2 novel clinically significant *RHO* mutations among 48 (2/48, 4,16%) probands is not concordant with earlier estimates of 16% to 28,5% of frequency among caucasians with adRP. [47, 49, 51-52] However, there is strong evidence for ethnic variations in the mutation frequency of *RHO* [53-56], as it has been observed with other genetic eye diseases, namely Leber Congenital Amaurosis; this could be the result of a relative geographic isolation for many centuries or due to the high consanguineous rate in our population and the fact that *RHO* gene mutations are primarily related to AD forms of RP. Although we are dealing with a relatively small patient population, it would be expected to find a higher percentage of mutations.

Both mutations affect highly conserved amino acid residues and are not present in the healthy control population. Some insight into possible mechanisms responsible for the ensuing retinal degeneration may be derived from considerations regarding the amino acids affected by these mutations.

The c.180 T>C transition (exon 1), leads to a substitution of a tyrosine, which is an aromatic amino acid with nonpolar and hydrophobic characteristics, for a histidine, which is a basic amino acid with polar (positively charged) and hydrophilic characteristics, might alter drastically the structure of the protein and the stability in the bilayer lipid membrane. Histidine has quite unique structure and functional properties sharing no resemblance with other amino acids. It is rather ambiguous whether it prefers to be buried in the protein core or exposed to solvents. Also, histidine is the most common amino acid in protein functional centers and binding sites, which could explain why the change may potentially render inadequate rhodopsin activity.

The c.207 C>T transition (exon1), leads to a substitution of an arginine, which is a basic amino acid with polar (positively charged) and hydrophilic characteristics, for a cysteine, which is a neutral and small amino acid, probably disturbing the structure of the first intradiscal loop and consequently the tertiary structure of the mutant rhodopsin. Cysteine is known to be frequently involved in disulphide bonds that stabilize the protein structure, especially important in extracellular domains; however, in this case it may still be involved in the formation of disulfide bonds and/or protein interactions. In the intracellular environment, cysteines may still play a key structural role. Their sulfydryl side-chain is excellent for metal-binding, such as zinc, thus compromising protein function.

According to disease mechanisms described in adRP [57], the novel mutations described here, belong to class II (defective protein folding) the most common in adRP mutations. Class I mutants, affecting the c-terminal region, fold normally in cell cultures but are not correctly transported into the outer segments *in vivo*. [57] Bioinformatic analysis and crystallography studies give further insight into the functional consequences of amino acid substitutions.

To our knowledge, only three mutations were identified nearby. The p.Thr58Arg [58-59] (Fig.12), a cytosine-to-guanine (C-to-G) transversion mutation in the second nucleotide of codon 58 of the RHO gene, causing a substitution of the amino acid arginine for a threonine, showed regional predilection for pigmentary changes in the inferior and inferonasal quadrants of the retina, as well as visual scotomas predominantly in the superior hemifields (sector RP). This clearly differs from the phenotype observed in our affected probands.

The p.Gln64ter mutation (Fig.12) [60] represents a nonsense mutation that is able to cause adRP, suggesting that synthesis of a rhodopsin fragment consisting of the

first 63 amino acids damages the rod photoreceptors. Cellular damage could result from disruption of the lipid bilayer structure or from interference with the folding or transport of other proteins.

The in-frame 12-bp deletion of codons 68 to 71 [48] occurs in the cytoplasmic loop connecting the first and second transmembrane helices (Fig. 12). This is the most conserved region on the cytoplasmic surface and has been suggested to be a point of interaction with cytoplasmic proteins. [61] However, it seems possible that the removal of these amino acids has an effect on protein folding in addition to any functional significance this region may have in the signal transduction pathway.

Rhodopsin mutations have been reported in association with other retinal phenotypes. Autosomal recessive Retinitis Pigmentosa (arRP) [8-11] is caused by mutations in the cytoplasmic and extracellular domains, what might suggest that they have a more damaging effect compared with mutations in the transmembrane domains. Congenital stationary night blindness (CSNB) [12-15] has also been described, as the result of mutations in the extracellular end of the second and seven transmembrane domains, strengthening the hypothesis described above. The two novel mutations described here, are located in the cytoplasmic end of the first transmembrane domain and in the first intracytoplasmic loop; this may underlie the early observed disease onset in our probands.

Suspicion of autosomal dominant pattern of inheritance usually occurs in the presence of mild sporadic cases [27]. In 10 to 40 percent of all cases of retinitis pigmentosa, only one person in a family is affected - simplex case. It can be difficult to determine the inheritance pattern in those cases because affected individuals may have no affected relatives or may be unaware of other family members with the disease. Simplex cases can also result from a new gene mutation that is not present in other

family members. Multiplex cases correspond to 2 or more affected family members (typically siblings) who have no pre-existing family history, which seems to be the case of patient MBC. Segregation analysis is still pending to confirm the etiology of our finding.

Although the typical manifestations present between adolescence and early adulthood, the age of onset has been documented to range from infancy to adulthood [62]. Due to the remarkable variation in how aware individuals are of their visual loss, the age of onset of symptoms is an imprecise measure of disease severity and gives little or no indication of when photoreceptor degeneration actually begins. [43]

Both probands report early-onset night vision disturbances accompanied by progressive loss of peripheral visual field. Photophobia became a problem after several years. There is no history of consanguinity or retinopathies. Clinically, they presented typical features of RP, including retinal vessels attenuation, bone spicule deposition, and a waxy appearance of the optic disc. Usually early-onset and severe forms of RP with myopia in male are associated with X-linked RP (XLRP). [27] Perhaps, the position of the substituted amino acids in the protein, and the side chain polarity of the substituted amino acids may explain the similarity of phenotypes.

AdRP, in most cases, is a long lasting disease that typically evolves over several decades with good overall long-term prognosis. Even at terminal stages, the disease progression remains slow. [27] The phenotypic variability seen between probands at age 50 could be explained by differences in genetic background or by environmental factors, even though both of them are of European decent and have comparable lifestyles.

This study contributed to emphasize the importance of RHO mutation screening in patients with RCD, since we identified two novel missense mutations. Also it gives

an overview of its prevalence in a Portuguese population. It was possible to attest the phenotypic variability associated with rhodopsin mutations and the need to improve our understanding of disease mechanisms to offer genetic counseling.

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