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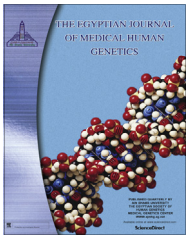

ORIGINAL ARTICLE

Rhodopsin mutations are scarcely implicated in autosomal recessive retinitis pigmentosa: A preliminary study of Egyptian retinitis pigmentosa patients

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Abstract *Background:* Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of inherited retinal degenerations that is estimated to affect more than 1.5 million people worldwide. RP is characterized by retinal pigment deposits visible on fundus examination, abnormal electroretinogram and progressive retinal dysfunction.

Aim: The present work aimed to identify the possible mutations in the rhodopsin gene (*RHO*) among Egyptian RP patients as well as identifying the different inheritance patterns of those patients.

Subjects and methods: Thirty diagnosed retinitis pigmentosa patients were enrolled in the study. Inheritance forms of RP were identified by recording full family history, the coding regions of the rhodopsin gene were sequenced using blood-derived genomic DNA samples donated by patients and fifteen healthy controls.

Results: A high percentage of autosomal recessive cases was reported. Also a high parental consanguinity rates were evident. Sequencing of rhodopsin gene revealed no mutations among the study population.

Conclusion: Rhodopsin mutations are scarcely associated with the autosomal recessive RP, suggesting that wide scale studies are needed to determine the genetic variations involved in RP and particularly in the autosomal recessive inheritance.

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1. Introduction

RP is the most common form of inherited retinopathies with a wide variety in gene involvement, the frequency of RP is approximately 1 in 3000 individuals [1,2]. To date up to 60 causative genes/loci have been identified in RP [24]. These genes are involved in various metabolic pathways including components of the photo-transduction cascade, proteins involved in the retinoid cycle or photoreceptor structural proteins [5,7,19].

Most genes for RP cause only a small proportion of cases, with the exception of the rhodopsin gene (*RHO*), which accounts for 25–30%, of autosomal dominant retinitis pigmentosa (adRP) patients [3,17,18]. The rhodopsin protein is a photon detector for low luminance conditions as it is responsible for the initiation of the visual transduction cascade upon the incidence of a photon of light [6,20]. Rhodopsin is primarily expressed in the rod photoreceptor cells, where it is packaged into membranous disks located in the rod outer segment region at high concentrations giving the rod cell a low threshold of sensitivity of single photons of light [4,25].

Rhodopsin is considered the most mutant protein causing the disorder with more than 100 different mutations [1,25,30]. Genetic counseling is of major importance in order to determine the inheritance pattern of the disorder and consequently the underlying genetic cause. Most *RHO* mutations are point mutations; $Pro^{23}His$ was the first identified mutation in adRP patients in North America but these point mutations vary among different ethnic groups [12,17]. Furthermore, many *RHO* mutations have been identified in only one family [10]. Rhodopsin gene mutations may lead to protein misfolding mediated neuro-degeneration either by causing misfolding of the protein or by interfering with the normal trafficking of the protein to the photoreceptor outer segment [21,22].

This study aimed to identify the associated mutations in the rhodopsin gene that leads to retinal degeneration in Egyptian RP patients. This might help understanding the genetic causes of photoreceptor degeneration, hoping to pave the way for future therapeutic approachs.

2. Subjects and methods

2.1. Human subjects

Thirty patients with RP were diagnosed using established ophthalmologic criteria that are based on fundus examination and electroretinogram (ERG). With the exception of patients with syndromic forms, all referred patients with RP were enrolled in this study regardless of the age of the onset. Fifteen controls were also enrolled in the study. All patients and controls gave their informed consent before participation in the study. We have taken consent of the Ethics Committee of Cairo University and the work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans.

2.2. Genetic counseling

Patients were referred to the Ophthalmo-genetics Department, Research Institute of ophthalmology. Thorough family histories were obtained from all patients and controls. Family pedigrees were constructed and accordingly patients were categorized either as sporadic where there is no other affected family member or as autosomal dominant, autosomal recessive or X-linked forms [9]. The criteria for autosomal dominant transmission were either the presence of three or more generations with both males and females among all affected family members, or at least two affected generations with male to male transmission [12,26]. For autosomal recessive transmission the trait appears mainly in sibs much more its appearance in parents or offspring. On average 25% of the sibs of the proband are affected if both parents are carriers. An autosomal recessive disorder is revealed by the appearance of the disorder in the male and female progeny of unaffected persons [29]. X-linked inheritance is characterized by expression of the trait by all males who carry the gene, but females are affected only if they are homozygous. None of the sons of an affected male are affected, but all his daughters are carriers [9,29].

2.3. Extraction of genomic DNA

Genomic DNA was extracted from 300 µl of peripheral blood lymphocytes using wizard genomic DNA extraction kit (promega, USA) according to manufacturer's protocol.

2.4. PCR

Exons of the *RHO* gene were amplified for each study subject using 5 primer pairs (primer sequences are listed in Table 1) [8]. PCR was performed in a reaction volume of 25 µl containing 1 U Taq polymerase, 0.2 µl of each dNTP, 2.5 µl of PCR buffer and 0.4 pmol/µl of each primer. A known volume of template DNA was added depending on the sample DNA concentration. On average, 4 µl of the template DNA was used. The temperature cycle was an initial denaturation of the DNA template at 94° C for 4 min, followed by denaturation during cycling at 94° C for 30 s for exons 1, 3, 5, for 45 s for exon 4, and for 1 min for exon 2. Annealing at 58° C was performed for 45 s for exon 4, for 30 s for exon 1, and for 1 min for exon 2. Annealing for exons 5, 3 was performed at 60° C for 30 s. Primer extension for all exons was at 72° C for 1 min. Denaturation, annealing and extension were repeated for 35 cycles in an automated thermocycler (BIOER Technology Co. Ltd.). An aliquot of 3 µl of PCR products was used to check the quality of the reaction products by agarose gel electrophoresis.

2.5. Purification of PCR products

QIAquick PCR purification kit protocol (QIAGEN) was used to purify DNA fragments from the PCR reaction. Three micro liters of the purified DNA was used to test the quality of the purified DNA by agarose gel electrophoresis.

2.6. Direct DNA sequencing

Purified PCR products were bi-directionally sequenced on an automated ABI PRISM (310 Genetic Analyzer) sequencer using ABI PRISM Big Dye terminator cycle sequencing ready reaction kit (PE applied Biosystems, USA).

Table 1 PCR primers used for amplifying the rhodopsin gene.

Exon	Primer sequence 5′ to 3′	Product size (bp)
Exon 1	AGC TCA GGC CTT CGC AGC AT GAG GGC TTT GGA TAA CAT TG	558
Exon 2	GAG TGC ACC CTC CTT AGG CA TCC TGA CTG GAG GAC CCT AC	290
Exon 3	CTG TTC CCA AGT CCC TCA CA CTG GAC CCT CAG AGC CGT GA	260
Exon 4	CAG CAT GCA TCT GCG GCT C CCT GGG AAG TAG CTT GTC CTT	384
Exon 5	CAC TAA CGT GCC AGT TCC AAG C TGA CTT CGT TCA TTC TGC ACA G	289

3. Results

3.1. Human subjects

Clinical diagnosis of RP patients was based on lesions in the fundus and reduced ERG. Patients showed intra-retinal pigmentation and decreased visual acuity that deteriorated in some patients to even no perception of light. Electroretinogram (ERG) represents the electrical response of the retina to a light stimulus and is used for the diagnosis of various retinal diseases. Dim flash ERG is performed on a dark-adapted eye, so the response is primarily from the rod system. Whereas flash ERG is performed on a light adapted eye and reflects the activity of the cone system [28]. Examination with ERG varied from a reduction in rod and cone response amplitudes to even undetected photoreceptor response (Fig. 1). Photopic ERG (which measures the cone response) was rather less affected than scotopic responses (which measure the rod response).

3.2. Genetic counseling

According to criteria mentioned in subjects and methods section, patients were classified into 3 groups: eight were sporadic (26.7%), five patients were autosomal dominant (16.6%) and 17 were autosomal recessive cases (56.7%). No case showed X-linked inheritance. Consanguinity was evident in 20 cases (66.6%); sixteen of them were autosomal recessive and four were sporadic. Table 2 summarizes the characteristics of the affected individuals.

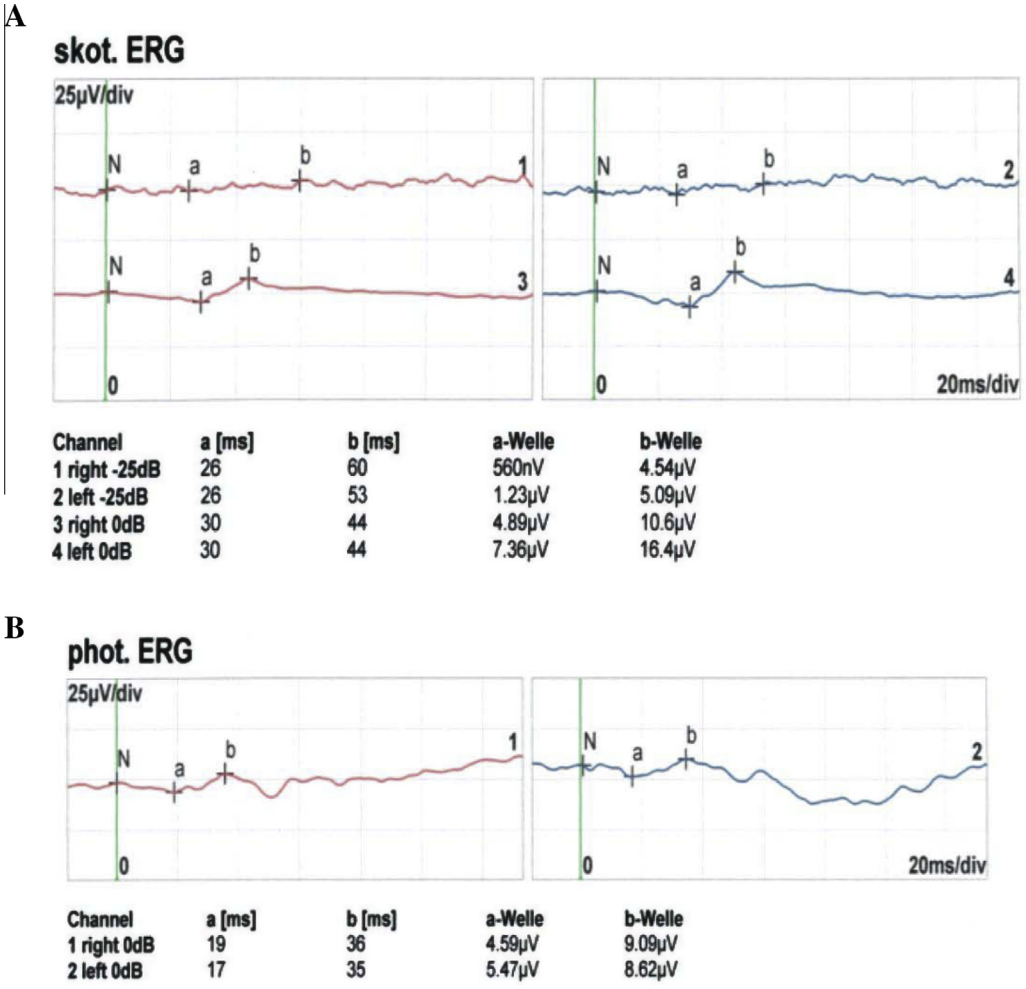

Figure 1 Flash ERG of RP patient. (A) Severely reduced scotopic responses, indicating markedly impaired rod function. (B) Photopic ERG is rather less affected than scotopic responses.

3.3. Sequence analysis

Rhodopsin exons were amplified using PCR. The PCR products showed well defined bands. Direct forward and reverse sequencing of the patients' *RHO* exons revealed normal sequences when compared to the gene bank sequence (GenBank NT_005612.16).

4. Discussion

Gene mapping and gene discovery have revealed that the molecular genetic causes of RP are unusually complicated especially in the autosomal recessive cases. Mutations in 22 different genes are associated with autosomal dominant RP (adRP) [27,30], whereas over 30 genes and loci have been implicated in autosomal recessive RP (arRP). Most of the genes associated with arRP are rare, causing 1% or fewer cases [30]. However, in the present study no mutations were detected in the examined *RHO* gene. This may be attributed to the high percentage of the autosomal recessive inheritance which is the most prevalent inheritance pattern in Egypt [13]. The high prevalence of recessive inheritance might be related to the high parental consanguinity rates as consanguinity is known to increase the frequency of recessive disorders [14]. Consanguineous marriage in Egypt is still high (35.3%) [15], the parental consanguinity rates among RP patients were much higher (71.4%) [13]. In the present study the parental consanguinity rates were 66.6% of the study population.

Although there are over 100 mutations in the *RHO* gene associated with RP identified in previous studies; only a few of them are inherited with the autosomal recessive pattern [30]. An up to date study aiming at determining the prevalence of *RHO* mutations in adRP Spanish families found that *RHO* mutations accounted for 21% of the study population, with the most prevalent mutation Pro347Leu responsible for 4.5% of all mutated adRP families [23].

Another study focused on the genetic heterogeneity of arRP and studied the gene coding for the β-subunit of the rod phosphodiesterase (PDEB), rhodopsin, peripherin/RDS and the gene coding the rod outer segment membrane protein 1 (ROM1) as well as two loci. Three homozygous mutations in the PDEB gene were found, accounting for 6% of all cases. No other disease-causing mutation was observed, suggesting that it is unlikely that these genes and loci account for a considerable proportion of arRP cases [31].

Also a previous study showed no mutation causing an amino acid substitution of *RHO* in 68 Japanese patients with RP [11]. In another attempt to identify *RHO* mutations associated with RP in Spain, thirty-six unrelated RP patients were

Table 2 Illustrates the personal data, inheritance forms and clinical data of the patients.

Patient ID	Age	Sex	Age of diagnosis	BCVA-RT	BCVA-LT	Inheritance pattern	Parental consanguinity
Code.1	33	M	Adulthood	1/60	1/60	Sporadic	+
Code.2	51	M	Adulthood	6/12	6/18	Sporadic	+
Code.3	38	M	Adulthood	6/60	1/60	adRP	–
Code.4	33	M	Adulthood	6/18	1.5/60	adRP	–
Code.5	32	M	Childhood	CF	1/60	adRP	–
Code.6	25	M	Childhood	5/60	3/60	adRP	–
Code.7	23	F	Adulthood	6/24	6/60	arRP	+
Code.8	55	F	Adulthood	No PL	HM	Sporadic	–
Code.9	25	M	Adulthood	2/60	CF	arRP	+
Code.10	40	F	Adulthood	CF	CF	adRP	–
Code.11	19	M	Adulthood	1.5/60	6/36	arRP	+
Code.12	30	M	Childhood	6/24	HM	arRP	+
Code.13	17	F	Adulthood	1/60	1/60	arRP	+
Code.14	32	M	Childhood	CF	1/60	arRP	+
Code.15	27	M	Adulthood	1/60	1/60	arRP	–
Code.16	30	M	Adulthood	5/60	3/60	arRP	+
Code.17	16	F	Childhood	6/24	6/60	arRP	+
Code.18	53	M	Childhood	2/60	3/60	Sporadic	–
Code.19	5	M	Childhood	HM	6/60	arRP	+
Code.20	47	F	Adulthood	2/60	2/60	arRP	+
Code.21	46	F	Adulthood	6/36	6/36	arRP	+
Code.22	6	F	Childhood	HM	HM	arRP	+
Code.23	5	F	Childhood	2/60	3/60	Sporadic	+
Code.24	31	M	Childhood	CF	HM	Sporadic	–
Code.25	50	F	Childhood	CF	CF	arRP	+
Code.26	52	M	Adulthood	6/60	HM	Sporadic	–
Code.27	47	M	Adulthood	No PL	HM	Sporadic	+
Code.28	39	F	Adulthood	3/60	3/60	arRP	+
Code.29	55	F	Childhood	2/60	CF	arRP	+
Code.30	32	M	Adulthood	6/24	HM	arRP	+

The table shows the sex, age of diagnosis, visual acuity, inheritance patterns and parental consanguinity. adRP: autosomal dominant retinitis pigmentosa; arRP; autosomal recessive retinitis pigmentosa; BCVA: best corrected visual acuity at time of examination; RT: right eye; LT: left eye; CF: count fingers; HM: hand movements; PL: perception of light.

screened for point mutations in the *RHO* gene. Neutral variations were found, that do not represent a change in the protein [16].

4.1. Conclusion

RHO gene mutations are very rare in autosomal recessive RP, so that more patients need to be screened in future studies keeping in mind that rhodopsin is not the only gene implicated in RP, but a much more variety of genes are also involved and need to be analyzed, especially in the autosomal recessive form.

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