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ORIGINAL PAPER

Optical coherence tomography and electro-oculogram abnormalities in X-linked retinitis pigmentosa

Enzo Maria Vingolo · Maria Luisa Livani · D. Domanico · Regina. H. F. Mendonça · Eduardo Rispoli

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Abstract
Purpose To determine the correlations between morphological optical coherence tomography (OCT) and electrophysiological electro-oculogram (EOG) alterations in families with X-linked retinitis pigmentosa (XLRP).
Design Observational case series.
Participants and Methods About 32 eyes of 16 members of four different families: Seven obligate carriers, four affected male homozygotes and five unaffected females underwent ophthalmologic completed exams including EOG and OCT. All the subjects were previously tested with genetic analysis. The results were statistically analysed.
Results The abnormalities in OCT were detected in all carriers and affected males consisting of macular edema and increased RPE reflectivity compared to no alterations in unaffected females. The EOG was flat in all affected males; distinctly abnormal in eight eyes of obligate carriers; normal in two eyes of obligate carriers and in all unaffected females. In two obligate carriers, the EOG was not performed due to a nuclear cataract. The correlations between OCT and EOG alterations were statistically significant.
Conclusions The OCT and EOG were demonstrated to be useful methods to identify the minimal alterations in carriers of X-linked retinitis pigmentosa.

Keywords Electrophysiological tests · Optical coherence tomography · X-linked retinitis pigmentosa

E. M. Vingolo (✉) · M. L. Livani · D. Domanico · R. H. F. Mendonça · E. Rispoli
Department of Ophthalmology, Inherited Retinal Diseases Unit, University of La Sapienza, Via Dandini 5, 00154, Rome, Italy
e-mail: evingolo@rdn.it

Introduction

Retinitis pigmentosa consists of a group of inherited retinal degenerative diseases that affect photoreceptors and retinal pigment epithelium [1, 2] Although for some authors X-linked retinitis pigmentosa (XLRP) can be considered a common disease form [3], for others, it is the least common [4]. In any case, it's the most severe genetic subtype of RP [2–6]. The XLRP produces a severe retinal degeneration in affected males but shows greater variability in female carriers [5]. According to hypothesis proposed by Lyon, carriers of an X-linked trait would be expected to exhibit greater variability because early in embryonic development, one x-chromosome, independently in each cell of a female, becomes inactive. As a consequence the tissue of a heterozygote female becomes a mosaic of cells with some cells expressing a normal and others expressing an abnormal gene [7].

The refined investigation on female carriers may give insights into pathogenic mechanisms involved in this disease [8]. Different investigative methods were used such as psychophysical tests showing differences between the carriers and normal subjects [9].

Multifocal Electroretinogram (mfERG) may prove to be a useful method for the evaluation and monitoring of localized retinal cone dysfunction. The mfERG can be abnormal in carriers, even in the presence of normal full-field ERG recordings. The full-field ERG does not allow resolution of the responses from discrete retinal areas, and small loci of retinal dysfunction may not alter the full-field ERG.The mfERG demonstrated patchy areas of retinal dysfunction in some carriers of XLRP with normal full-field ERG (amplitude and implicit time) [4].

The difference in foveal reflectance between carriers and normal, suggests a difference in the density of macular pigment [2]. In order to determine an efficient method in order to study XLRP families, we decided to study the correlations between morphological and functional alterations using the optical coherence tomography (OCT) and the electrophysiological tests (mainly the EOG).

Methods

Sixteen members of four different families: Seven obligate carriers, four affected male homozygotes, and five unaffected females underwent ophthalmologic completed exams including ERG, EOG and OCT. All the subjects were examined in the ambulatory of Electrophysiology of the Ophthalmic Clinic of the Faculty of Medicine at the ''Università degli Studi La Sapienza''—Rome and were previously tested with genetic analysis.

In analyzing the EOG responses we considered the Arden's ratio (RA): light peak (LP)/dark through (DT) × 100% [10].

The dark period (with a luminance of the adapting field of 0 Cd/m^2) were long 15 min and followed by a fast increase of the luminance of the adapting field until 400 Cd/m^2. The light period were long 15 min to. We assume that 210% is the lower limit of normal [10] and the mean normal value of 250 as average ± 2 Standard Deviation is ± 55 [11].

For the ERG (performed with METROVISION ERG MF1 Clermond-Ferrand France), we used the ISCEV protocol and we considered 'rod response', 'mixed response' and 'cone response').

The OCT results were classified in points based on intensity alterations as follows:

- Zero classifies as normal.
- One point if increase RPE reflectivity is shown (0,5 if mild and 1 if marketed).
- Two points if decrease of outer layer reflectivity is shown (one point if mild, two points if marketed).
- Two points if decrease (or increase) of retinal thickness is shown (1 point if mild and 2 points if marketed).
- 2 points if decrease of photoreceptor outer and inner segments is shown (only if marketed).

Was used a 'OCT 3 stratus' (ZEISS-MEDITEC USA). Photoreceptor outer and inner segment decrease was determined with two cursors of the 'retinal thikness analysis'.

Statistical analysis

A descriptive analysis was carried out, consisting of the calculation of the mean and standard deviation.

Having observed an abnormal distribution of the data gathered, we used non-parametrical statistical tests. Having considered the scoring items of OCT independent we proceeded to compare the mean of the results from the two exams (OCT and EOG) in the three groups (obligate carriers, unaffected females and patients with XLRP) with the Kruskal–Wallis anova. The comparison for each two groups was made with Mann–Whitney test. As we routinely do, we set a type 1 risk of error (alpha) equal to 0.05.

Finally, we calculated the non parametric correlation coefficient of Spearman and the significance level.

The BMD Release Seven program (1993) was used for processing.

This series of dates was not amenable to have CUT OFF dates, to draw a curve of ROCK and to have an appropriate grading scale.

Results

In the present study 100% of the carriers had fundus abnormalities like a tapetal reflex, characterized by pigmentary irregularities associated to EPR deficits and retinal flecks as shown in Fig. 1.

All XLRP male affected patients presented RP typical fundus alterations diffuse for 360 as shown in Fig. 2. Non-carrier females presented normal fundus appearance.

Abnormalities in OCT were detected in all carriers and affected males consisting mainly of macular edema and increase RPE reflectivity, compared to no alterations in unaffected females (Table 1). As we can see, the OCT alterations were much more severe in XLRP male patients (4 and 5 points) in comparison with carriers (1–3 points). Figure 3 shows an example of one XLRP patient classified with five points.

The EOG was abnormal in all affected males; distinctly abnormal in eight eyes of obligate carriers; normal in two eyes of obligate carriers and in all unaffected females (Table 1). In two obligate carriers, the EOG was not performed due to a nuclear cataract. The lowest recorded EOG ratio in obligate carriers was 115%.

Reduced rod and cone ERG were present in all obligate carriers. Typical fundus alterations with non-recordable ERG was noted in 100% of XLRP patients. Normal responses were obtained in all unaffected females. Figure 4 shows the correlation between 'b' wawe amplitudes of ERG (rod, mixed and cone response) and OCT (evaluation of photoreceptor layer thickness) alterations.

Experimental results from tests conducted on the EOG and OCT were taken into consideration and compared between groups. During a first

Table 1 The EOG and OCT results of right eye (RE) and left eye (LE)

	EOG RE	EOG LE	OCT RE	OCT LE
Carriers	180%	166%	2	3
	195%	250%	1	1
	130%	115%	2	3
	231%	194%	1	2
	141%	137%	2	2
Unaffected	293%	310%	0	0
females	241%	270%	0	0
	235%	248%	0	0
	211%	265%	0	0
	280%	310%	0	0
XLRP	100%	100%	4	5
	100%	100%	5	5
	100%	100%	4	4
	100%	100%	5	5

The EOG response are in Arden's ratio: mean normal values 250%(+/–55% SD)

The OCT's are classified in intensity of alterations in as follows:

Zero classifies as normal

One point if increase RPE reflectivity is shown (0, 5 if mild and 1 if marketed)

Two points if decrease of outer layer reflectivity is shown (one point if mild, two points if marketed)

Two points if decrease of retinal thickness is shown (one point if mild and two points if marketed)

Two points if decrease of photoreceptor outer and inner segments is shown (only if marketed)

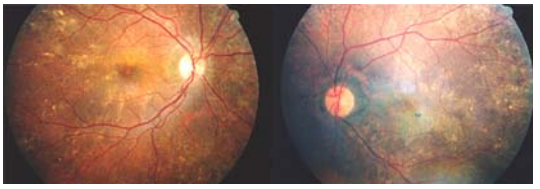

Fig. 1 Fundus of a carrier of XLRP (B.P.age 57 yrs)

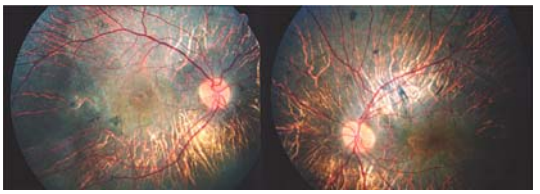

Fig. 2 Typical fundus of a male XLRP affected (T.A. age 31 yrs. son of the carrier showed in Fig. 1)

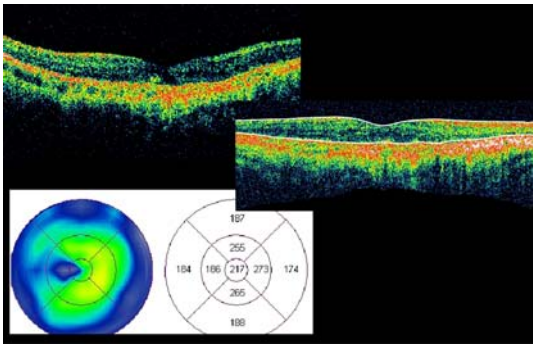

Fig. 3 OCT images of a XLRP male patient

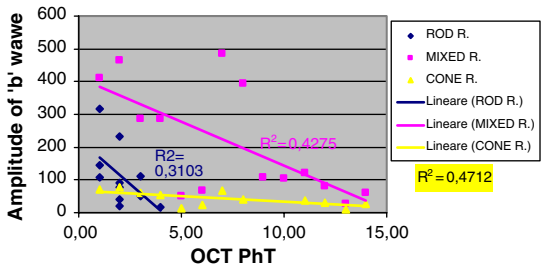

Fig. 4 Correlation's graphs between ERG data and OCT alterations

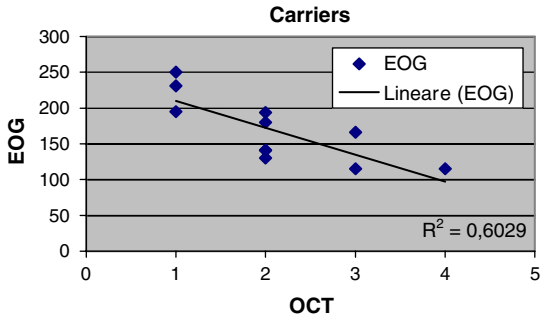

Fig. 5 graph showing correlation of OCT values for each obligate carrier against their Arden ratio (EOG)

stage, we compared values from OCT and EOG respectively in all subjects together (obligate carriers, unaffected females and patients with XLRP). A significant statistical difference between the three groups ($p = 0.0001$) was detected with both methods.

Similar results were obtained when comparing OCT and EOG between separated groups (obligate carriers/unaffected females), (unaffected females/patients with XLRP) and (obligate carriers and XLRP). These comparisons were statistically significant. Figure 5 shows a correlation of OCT values for each obligate carrier against their Arden ratio (EOG) values. We detected a very high inverse correlation coefficient in both diagnostic methods ($r = -0.937$; $p < 0.01$) so we can be certain that the OCT alterations parallel EOG alterations.

Discussion

It has been suggested that fundus abnormalities are detected in 77% of obligate carriers. Normal fundus with abnormal EOG and/or ERG was reported in 16% of obligate carriers [12]. Others have observed that fundus alterations in carriers are also important for visual prognosis counselling because carriers with only a tapetal-like retinal reflex had a better prognosis to retain visual function than those with peripheral retinal pigmentation [13].

Although many studies have demonstrated electrophysiological alterations in XLRP, unfortunately the EOG is not a commonly performed exam. In the present study, the EOG proved to be a useful method because the EOG was flat in all affected males, distinctly abnormal in four obligate carriers, and normal in all unaffected females (Table I). According to some authors, there was a tendency in carriers of RP to a subnormal EOG [14]. The EOG was not very useful, since almost 36% had a normal ratio even in cases with fundus abnormality [3]. Contrasting with our findings, in a study of 31 obligate carriers of XLRP, alterations to the EOG were detected in only 41% of the cases [12]. The incidence of EOG abnormality increased significantly ($p < 0.05$) in carries over 40 years of age compared with younger ones [12]. The EOG can be helpful in the evaluation of carriers with normal or equivocal fundi and equivalent ERG findings [12].

The ERG results can be used to suggest the nature of the retinal abnormality [15]. Many descriptions of the ERG alterations in carriers are reported. The ERG recorded using the brightest stimulus flashes has proved to be very effective for carrier identification [16]. Reductions in *b*-wave amplitudes of the carriers were equivalent for the rod and cone systems [16, 17]. However, this conclusion should not imply that all carriers had equivalent losses of function for the two photoreceptor systems [16]. For identifying carriers, the b-wave implicit times were found to be more informative than *b*-wave amplitudes [18]. In addition, in the present study, all obligate carriers presented a reduction in equivalent rod and cone ERG responses.

In one study of XLRP, non-detectable electroretinographic amplitudes in more than two thirds of the patients were demonstrated. In those with recordable responses, rods were more affected than cones in 50% of the patients, in the

other 50%, cones were less damaged than rods [6]. Some authors noted that in XLRP, both cone and rod responses were reduced in amplitude and also had a prolongation in implicit time, and that cone responses were detectable even after rod responses had disappeared [19]. The abnormality includes the a as well as the *b*-wave of both receptor systems and, therefore, must initially involve both photoreceptors cells [19]. The reduction in amplitude of cone responses in XLRP is accompanied by a prolongation of cone implicit time, a phenomenon not observed in the dominantly inherited disease [19]. Unfortunately, in the present work, all the affected patients had non-detectable ERG responses. In comparison with the literature, we can say that when our XLRP patients were examined, they already had a severe retinal involvement because of the absence of responses.

The preservation of the ocular electrical responses, in heterozygous females, suggests that the disease in women is qualitatively different from that in men and in other genetic forms of retinitis pigmentosa [3]. Comparison between female carriers and affected males suggests that the males have widespread retinal involvement, while females have loss only in specific areas [19].Our findings were in agreement with the literature. Non-detectable electroretinographic responses in affected male contrast with reduced rod and cone ERG responses in all obligate carriers. Abnormal ERG of carriers of XLRP contrasted with normal ERG in female carries of autosomal recessive disease. These results are important to establish the mode of inheritance [20].

A combination of electrophysiology and fundus examination identified 93% of obligate carriers, leaving 7% who could be identified only by family history [12]. According to other authors, approximately 86% of carriers can be identified by reductions in ERG, 87% can be identified by fundus change, and 100% can be diagnostic by fundus coupled with the ERG [8]. In our study, 100% of carriers had ERG's alterations, 80% abnormal EOG, and 80% fundus alterations. We believe that the combination of fundus examination and electrophysiological tests (EOG and ERG) are able to identify 100% of carriers.

The OCT permits the analysis of cross-sectional tomograms of the ocular tissue. With the OCT, we can detect reflection from the neural retina, pigment epithelium and choriocapillaris and also loss of pigment, retinal thickness, cystic spaces, exposure of underlying choroidal vessels, scattered aggregates of pigment and sparing of the macular area [21]. Every obligate carriers and patients with XLRP presented abnormal OCT. A significant statistical difference between carriers and affected males was detected, showing the capacity of the OCT to define the intensity of alterations. Normal results were obtained in all unaffected females. The OCT could be effective for clinical assessment [21]. The OCT alterations paralleled with those from EOG, showed by a very high inverse correlation coefficient in both diagnostic methods.

There is no doubt about the importance of the EOG in XLRP families. However, it is a test that is still lacking well-defined standards, especially for obligate carriers. To our knowledge, few studies [3, 12, 14] have given due importance to the EOG. We believe that abnormal EOG values can better define the carriers.

The ERG is a standardized clinical examination for these patients. In our study, the incidence of ERG abnormalities was 100% in obligate carriers. These findings could be important to detect the disease, that can then be confirmed with others examinations. The possibility of improved genetic and educational counselling in families afflicted by XLRP justifies the considerable diagnostic effort involved in ERG examinations [17]. Our results confirm the importance of ophthalmologic examinations as a powerful and crucial method for diagnosis XLRP. The use of the EOG and the ERG, including measurement of the rod response b wave latency, may increase the carrier detection rate [12].

Although XLRP occurs in approximately 8% of the families [8, 22], it is extremely devastating. Innovative investigation on female carriers of this disorder may afford insights into pathogenic mechanisms involved in this tragic affliction [8]. These observations are of general value for the diagnosis of this disease and for counselling of patients afflicted with this severe form of hereditary night blindness [6]. We believe that a

complete ophthalmological examination especially with fundus, ERG, EOG and OCT is important to define this pathology with more precision moreover the present data support the EOG as a satisfactory electrodiagnositic test for distinguishing carriers of XLRP from non-carriers.The possibility of a coherence between the morphological and electrophysiological exams emphasizes the importance of the EOG and OCT in XLRP families. Although our series was not amenable to draw conclusions, it was enough to suggest ideal exams to study XLRP patients.

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