Lacrimal Gland Abnormalities in Blepharophimosis, Ptosis and Epicanthus Inversus Syndrome

Catarina Xavier[1]; Ana Filipa Duarte[1]; Diana Antunes[2]; Maria Elisa Luís[1]; Miguel Boncquet Vieira[1]; Ana Xavier[1]; Francisco Trincão[1]; Alcina Toscano[1]
[1] Serviço de Oftalmologia, Centro Hospitalar Universitário Lisboa Central
[2] Serviço de Genética, Centro Hospitalar Universitário Lisboa Central

ABSTRACT

Introduction: Blepharophimosis, ptosis and epicanthus inversus syndrome (BPES) is an uncommon autosomal dominant congenital disease caused by a mutation in the forkhead box L2 *(FOXL2)* gene. Its major features are blepharophimosis, blepharoptosis, epicanthus inversus and telecanthus, and recently there have been some reports linking this disease to lacrimal gland (LG) agenesis. We studied the LG changes in BPES patients from a tertiary centre in Portugal.

Material and methods: Retrospective study of six patients with BPES, with ages between 1 and 39 years old. Lacrimal film evaluation was performed using slit-lamp biomicroscopy and Schirmer I test, and LG volume was measured on computed tomography scans. All patients were screened for *FOXL2* mutations.

Results and discussion: The lacrimal film was abnormal in 2 patients. Both presented a reduced lacrimal meniscus; in one patient the Schirmer I test was decreased and in the second patient a significative superficial keratopathy was visible. An absence of the LG was disclosed on both CT scans. In 2 other patients LG volume was bilaterally reduced and in 1 it was normal. The remaining 1-year-old child had visible LGs and a normal lacrimal status evaluation. In this group of patients, clinical changes found on the lacrimal film evaluation were greatly associated to LG dysgenesis. A *FOXL2* gene mutation was found in all cases, one of them not previously described.

Conclusion: This study reinforces the recently described association between BPES and alacrimia and the importance of a detailed lacrimal evaluation in these patients, specially if they are surgical candidates. A new *FOXL2* mutation not previously described is also reported.

Keywords: Blepharophimosis, BPES, alacrimia, FOXL2, lacrimal gland agenesis

INTRODUCTION

Blepharophimosis, ptosis and epicanthus inversus syndrome (BPES) is an uncommon congenital disorder characterized by soft tissue developmental abnormalities, namely blepharophimosis, blepharoptosis, epicanthus inversus and telecanthus.[1-4] Additional features include lateral lower eyelid ectropion, hypertelorism, hypoplasia of the superior orbital rims, abnormalities of the lacrimal drainage system, angle dysgenesis, iris-retinochoroidal colobomas, optic nerve hypoplasia and higher incidences of strabismus.[1,2] There are two types of BPES: type I includes

the four major features associated with premature ovarian insufficiency in women; type II includes only the four major features.[1,5]

BPES is caused by a *forkhead box L2* (*FOXL2*) gene mutation on chromosome 3q23 that has usually an autosomal dominant inheritance, but it can also be the result of a *de novo* mutation.[2] More than 90 pathogenic/likely pathogenic mutations have been previously reported, 70% of them being intragenic mutations, including premature stop codons, missense mutations, expansions of the region encoding the poly-Ala tract and frameshift mutations leading to a shorter or longer protein,[6] resulting in the lack of *FOXL2* expression in the developing periocular tissues and ovarian follicular cells.[2]

Absence of tear production has not traditionally been described in cases of BPES, but recently there have been some case reports linking alacrimia to BPES.[1,2] In 2017 *Duarte et al* showed that the association between alacrimia and BPES is not incidental. They described a significant number of patients with reduced or absent LGs and suggested an association between the type and location of the mutation and LG abnormalities.[7]

In this study we analysed changes in the lacrimal secretor apparatus in 6 BPES patients from our institution, as well as their *FOXL2* gene for a possible genotype-phenotype correlation.

MATERIAL AND METHODS

Six patients with BPES, followed in the Ophthalmology Department of Centro Hospitalar Universitário de Lisboa Central between 2016 and 2018, were studied. Informed consents were obtained from the patients or their parents when underage. These patients were divided into 2 groups depending on their age: group 1 with > 18 years old (39, 36 and 21 years old) and group 2 with <18 years old (8, 5 and 1 years old). None of the patients took medications known to interfere with the lacrimal secretion.

A complete ophthalmologic examination was performed, with a particular emphasis on the evaluation of the lacrimal film by slit-lamp biomicroscopy, including lacrimal meniscus height and corneal surface staining using fluorescein. Schirmer I test was also performed on cooperative patients (without anesthesia - a score <10 mm after 5 minutes with the eyes closed was considered as indicative of a reduced aqueous tear production). An orbital CT scan with 2mm slices protocol was performed, and the LG volume was measured using Osirix software, as described by *Bingham et al.*[8] Briefly, the LG area was measured on consecutive axial slices of soft tissue window orbital CT scans, which were added in order to obtain a final volume. According to *Bingham et al* results,[8] obtained for >18 years old persons, LG volumes values <0,314 cm^3 on the right orbit and <0,307 cm^3 on the left orbit were considered as reduced for patients from group 1.

For patients P1-P5 (table 1), genetic analysis was performed by PCR amplification and Sanger sequencing of all the coding regions, including *FOXL2* adjacent intronic regions. For patient P6, genetic analysis was done by Next Generation Sequencing by multigene panels tests (98 genes associated with blepharophimosis *HP:0000581 The Human Phenotype Ontology*).

RESULTS

Results are detailed on Table 1. We studied 6 patients from 4 different families, patients P1 and P4 and patients P2 and P5 were progenitor and offspring, respectively. Patients P3 and P6 didn't have affected relatives. All patients had congenital eyelid malformations corresponding to the four major features of BPES. Patient P3 had type I BPES with primary amenorrhea from early ovary insufficiency. Three patients were already submitted to corrective eyelid surgery, one of them counting a total of 6 surgeries (3 frontal suspensions of the right side and 2 of the left side).

In group 1, one patient (P1) showed clinical signs of reduced tear production bilaterally, presenting a reduced lacrimal meniscus height and a severe decrease of Schirmer I test result. The remaining patients (P2 and P3) didn't have objective lacrimal film changes. Regarding LG volume, one patient (P1) showed bilateral absence of the lacrimal glands on CT scan, and in the remaining 2 patients the glands were visible on imaging, one of them (P2) having a reduced volume bilaterally (OD: 0.12 cm^3; OD: 0.14cm^3) and the other (P3) a normal volume (OD: 0.59 cm^3; OS: 0.69 cm^3).

In group 2 there was also one patient (P4) who showed clinical signs of reduced lacrimal production, namely a bilateral reduction of the lacrimal meniscus height and a moderate/severe diffuse superficial keratitis. The other patients (P5 and P6) didn't show clinical signs of reduced tear production. The Schirmer I test wasn't performed on this group due to lack of cooperation. On CT scans, one

patient (P4) had a bilateral absence of LGs. Patient P5 presented LGs volumes of 0,06 cm^3 on the right and 0,16 cm^3 on the left. In the remaining patient (P6), LGs measured 0,29 cm^3 on the right and 0,22 cm^3 on the left.

The molecular analysis of the *FOXL2* gene showed pathogenic variants of the gene on all tested patients. Four different mutations were found, one in each family. In patients P1 and P4 a missense mutation was detected, c.313A>C, in the DNA-binding forkhead domain of the gene. Patients P2 and P5 had an in-frame duplication of 30 nucleotides, c.672_701dup30, resulting in a polyalanine expansion. Patient P3 had a nonsense mutation, c.645T>G, that resulted in a premature STOP codon. This was a *de novo* variant, not present in the progenitors. Patient P6 had a microdeletion including *FOXL2* gene, c.(?_-1)_(*1_?)del, resulting in no protein (p.?). This one is probably also a *de novo* mutation because his parents are clinically unaffected, although not genetically studied.

Table 1 - G, group; M, male; F, female; +, present; ↓ visibly decreased; Unc, uncooperative; *Comparative analysis to normal LG volume:[8] Reduced when below and normal, when above the minimum value measured in the control group; ?, no control group; NM, not measurable;

G	Patient	Age (years)	Gender	Number of previous surgeries	Laterality	Lacrimal film evaluation			Lacrimal gland imaging		FOXL2 molecular analysis		
						Lacrimal meniscus	Schirmer I (mm)	Superficial keratopathy	Glandular volume (cm^3)	Lacrimal gland size rating*	cDNA	Protein	Type
1	P1	39	M	None	OD	↓	3	N	NM	Absent	c.313A>C	p.N105H	Missense
					OS	↓	4	N	NM	Absent			
	P2	36	M	2	OD	+	22	N	0.12	Reduced	c.672_701dup30	p.A224-A234dup	In-frame Duplication
					OS	+	32	N	0.14	Reduced			
	P3	21	F	1	OD	+	18	N	0.59	Normal	c.645T>G	p.Tyr215x	Nonsense
					OS	+	36	N	0.69	Normal			
2	P4	5	F	None	OD	↓	Unc	Diffuse	NM	Absent	c.313A>C	p.N105H	Missense
					OS	↓	Unc	Diffuse +	NM	Absent			
	P5	8	M	6	OD	+	Unc	N	0.06	Reduced	c.672_701dup30	p.A224-A234dup	In-Frame Duplication
					OS	+	Unc	N	0.16	Reduced			
	P6	1	M	None	OD	+	Unc	N	0.29	?	c.(?_-1)_(*1_?)del	p.?	Microdel incl. *FOXL2*
					OS	+	Unc	N	0.22	?			

DISCUSSION

Alacrimia and lacrimal gland agenesis are rare. There have been only a few reports of complete absence of the lacrimal glands in literature.[1]

After 2 case reports of alacrimia in BPES patients, one with absence[1] and the other with the presence of the lacrimal glands,[2] *Chawla et al*[4] studied the gland status in 33 Indian BPES patients. Even though in none of the patients the lacrimal gland was absent in CT scans, glandular volume was not measured. In 2017, *Duarte et al* were the first to objectively evaluate LG volumes in BPES, in a study including 21 patients. In this report more than half of the patients had no measurable LGs (42.8% bilaterally and 9.5% unilaterally), and one third had reduced LG volumes.[7]

The present study favours this finding, describing 2 in 6 patients with no measurable LGs and other 2 with reduced LG volumes (we believe P5 LG volumes to be reduced, even without a control group), therefore reinforcing an association between LG agenesis/reduction and BPES. We cannot take any conclusion about the LG volume of the 1-year-old patient (P6) as data of normality was not found on literature. The only patient who clearly showed a normal LG volume had type I BPES. Our results also showed agreement between LG absence and the clinical evaluation of the lacrimal film, with changes being exclusively found in patients without measurable LGs on the CT scan. Patients with reduced lacrimal gland volumes seem to have a normal ocular surface and lacrimal film evaluation.

Regarding the genetic analyses, each family showed a different mutation. A missense mutation, c.313A>C, causing the substitution of an asparagine for a histidine in the forkhead domain of *FOXL2;*[9] a 30-nucleotide duplication (c.672-701dup30) causing an expansion of 10 alanine residues (p.A224-234dup) within the polyalanine tract,[10] already been reported in distinct families with different geographic and ethnic backgrounds;[7] a nonsense mutation, c.645T>G, causing a premature STOP codon; and a microdeletion including *FOXL2* gene, c.(?_-1)_(*1_?)del.[11] The c.313A>C mutation was the only one associated with LG

agenesis; the c.672-701dup30 was related to LG volume reduction and the novel c.645T>G with a standard volume LG. The c.(?_-1)_(*1_?)del was associated to the presence of LGs, which volumes we cannot characterize as reduced or normal due to the lack of a control group.

The c.645T>G mutation is a new mutation not previously reported in the literature. It results in a protein with 215 instead of 376 amino acids and was present in a patient with type I BPES. The literature corroborates this finding: pathogenic variants predicted to result in proteins truncated before the polyalanine tract (which starts at the 221 amino acid position) preferentially lead to premature ovarian insufficiency (BPES type I).[5]

Our study reinforces the relationship between BPES and lacrimal gland malformations and the importance of a careful ocular surface and lacrimal film evaluation in all BPES patients. Given the size of our sample, a genotype-phenotype association cannot be established, nevertheless, our results add some information that can be useful in future studies.

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CONTACTO

Catarina Xavier
Serviço de Oftalmologia
Centro Hospitalar Universitário de Lisboa Central
Alameda Santo António dos Capuchos
1169-050 Lisboa
E-mail: acmx1992@gmail.com

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