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Lacrimal Gland Involvement in Blepharophimosis-Ptosis-Epicanthus Inversus Syndrome

Ana Filipa Duarte, MD,[1] *Patricia M.S. Akaishi, MD,*[1] *Greice A. de Molfetta, PhD,*[2] *Salomão Chodraui-Filho, MD,*[3] *Murilo Cintra, MD,*[3] *Alcina Toscano, MD,*[4] *Wilson Araujo Silva, Jr., PhD,*[2] *Antonio A.V. Cruz, MD*[1]

Purpose: To describe the involvement of the lacrimal gland (LG) in blepharophimosis-ptosis-epicanthus inversus syndrome (BPES).

Design: Observational, cross-sectional study.

Participants: Twenty-one patients with BPES (10 female, 11 male) aged on average 15 years (range, 2−39 years), from 3 Brazilian medical centers and 1 Portuguese medical center.

Methods: Patients had their ocular surface evaluated with slit-lamp biomicroscopy, and tear production quantified with the Schirmer test I. The LG volumes were measured on computed tomography (CT) scans in the BPES sample and in a group of age-matched subjects imaged for nonorbital diseases. Sixteen patients were screened for mutations in the *FOXL2* gene.

Main Outcome Measures: Lacrimal meniscus height, Schirmer test I, presence of superficial punctate keratopathy (SPK), LG volume, and molecular analysis of the *FOXL2* gene.

Results: Absence of LG was detected bilaterally in 9 patients (42.8%) and unilaterally in 2 patients (9.5%). When considering only patients with measurable LG, the median volume was 0.22 cm^3 in the right eye (range, 0.06−0.36 cm^3) and 0.24 cm^3 in the left eye (range, 0.08−0.34 cm^3). These values were significantly lower than those for the age-matched controls (median = 0.54 right eye and 0.53 left eye; $P < 0.05$). There was a significant association between deficiency of tear production and LG volume reduction and agenesis. Molecular analysis of the *FOXL2* gene revealed the presence of 8 distinct mutations, 4 of them novel ones. A significant reduction of LG size or agenesis was associated with mutations affecting protein size (due to underlying changes in the stop codon location) or the DNA-binding forkhead domain (Fisher exact test, $P = 0.021$). In 3 probands, the underlying genetic defect was not found.

Conclusions: This is the first study reporting LG volumes in BPES, describing a significant number of patients with LG agenesis. The association between alacrima and BPES is not incidental, and a thorough evaluation of tear production is recommended especially if ptosis surgery is planned. *Ophthalmology 2016;■:1−8 © 2016 by the American Academy of Ophthalmology*

Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) (Online Mendelian Inheritance in Man 110100) is an uncommon disorder first described in 1889 by Vignes.[1] Three decades later, Dimitry[2] defined its inheritance pattern as autosomal dominant, which was confirmed over subsequent years. Its molecular basis remained undetermined until 2001, when Crisponi et al[3] finally identified forkhead box protein L2 (*FOXL2*) (Online Mendelian Inheritance in Man *605597) on chromosome 3q23 as the causal gene.[3] This single-exon gene encodes a forkhead transcription factor expressed in the developing eyelid mesenchyme and fetal and adult ovaries.[4,5] Thus, depending on its expression, there are 2 types of BPES: type I with premature ovarian failure and type II with normal ovary function.[6,7] Severe ptosis with poor levator function, epicanthus inversus, and telecanthus are present in both types. We describe a significant spectrum of lacrimal gland (LG) changes in BPES. In addition, molecular analysis of *FOXL2* was performed in 16 patients in an attempt to obtain new insights into a possible genotype−phenotype correlation.

Methods

Patients

The research adhered to the tenets of the Declaration of Helsinki. Approval was obtained from the Institutional Review Boards and Ethics Committees, and all subjects gave written informed consent to participate in the study.

Twenty-one patients with a clinical diagnosis of BPES were recruited from 1 Portuguese and 3 Brazilian medical centers. Mean age was 15 years (range, 2−39 years). Schirmer test I (without anesthesia) and slit-lamp biomicroscopy assessment of the lacrimal meniscus height and corneal fluorescein staining were performed in all patients. A Schirmer's test score <10 mm after 5 minutes with

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Table 1. Primers Used for Amplification and Sequencing of the Entire Coding Region of the *FOXL2* Gene

Primer Names	Primer Sequences (5'>3')	Size of the PCR Product (bp)	Annealing Temperature (°C)
FOXL2-1.1F	TTTGAGACTTGGCCGTAAGC	444	55
FOXL2-1.1R	TGATGAAGCACTCGTTGAGG		
FOXL2-1.2F	CGAAGTTCCCGTTCTACGAG	576	57
FOXL2-1.2R	CCAGGCCATTGTACGAGTTC		
FOXL2-1.3F	GTACACACGCGTGCAGAGC	407	57
FOXL2-1.3R	CGTCCCTGCATCCTCGCATC		

bp = base pair; PCR = polymerase chain reaction.

the eyes closed was considered as an indication of reduced aqueous tear production.[8,9] Two radiologists measured LG volumes on computed tomography (CT) scans using OsiriX software (developed by Pixmeo, SARL, Geneva; http://www.osirix-viewer.com) as the Digital Imaging and Communications in Medicine (DICOM) viewer. Briefly, as described by Bingham et al,[10] the LG area was measured from consecutive axial slices of orbital CT scans and added to obtain a final volume. The same protocol was applied to a group of 30 age-matched patients imaged for nonorbital pathology, which constituted the control group. The Wilcoxon signed-rank test was used to compare right and left LG volumes, and the Mann−Whitney test was used to compare LG volumes between groups. The Mann−Whitney *U* test was used to compare the Schirmer's test scores between eyes with nonmeasurable versus reduced LG. Finally, the Fisher exact test was used to analyze the association between the signs of keratopathy and LG size categorized as reduced and nonmeasurable. In all these tests, a *P* value ≤0.05 was considered significant.

DNA Amplification and Exon Sequencing

Sixteen patients were submitted to exon sequencing of the *FOXL2* gene. Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp DNA Blood isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For the analysis of the *FOXL2* gene, 3 pairs of primers were designed to cover the entire *FOXL2* exon and its splice site junctions (Table 1). The polymerase chain reaction−amplified DNA fragments were subjected to direct sequencing using the automatic capillary sequencing system ABI 3500X Genetic Analyzer (Applied Biosystems, Foster City, CA) and the Big Dye terminator v3.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions. The results were analyzed using the FinchTV version 1.4.0 software (Geospiza, Seattle, WA); the sequences obtained were compared with the reference from GenBank database (NM_023067), and the mutation nomenclature was used according to Human Genome Variation Society guidelines (http://www.hgvs.org/mutnomen). Pathogenic scores for missense mutations were calculated using the prediction tools SIFT,[11] MutPred,[12] and MutationTaster.[13]

Genotype−Lacrimal Gland Phenotype Correlation

To identify a possible genotype−phenotype correlation, 2 groups of mutations were considered: those affecting protein size or its functional domain and those not affecting the protein catalytic domain or its structure. Fisher exact test was used to determine the significance of the association between these 2 categories of mutations and LG abnormalities. A *P* value ≤0.05 was considered significant.

Results

Clinical and molecular data are shown in Table 2. Eight of the 21 patients were relatives belonging to 4 distinct families. Patients 1 and 2, 13 and 14, and 15 and 16 were progenitor and offspring, respectively, and patients 18 and 19 were twin brothers. Fifteen patients (71.4%) had already been submitted to some surgical procedure to correct their eyelid malformations. In 9 patients (42.8%), more than 1 procedure had been performed, including multiple revisions of silicone or autologous fascia slings for ptosis surgery. Twenty-two eyes (52.4%) from 12 patients showed clinical signs of reduced tear production manifested as decreased lacrimal meniscus height, low values on the Schirmer test, or superficial punctate keratopathy (SPK) of variable severity. Of these 22 eyes, 20 (90.9%) did not have a measurable LG ipsilaterally, and in the remaining 2 eyes, the LG size was markedly reduced.

A significant difference was found between the Schirmer's scores in absent versus reduced LG cases ($P = 0.0006$). Figure 1 shows the distribution of Schirmer's scores versus LG volumes. Eleven of the 14 eyes (78.6%) with absent LG showed Schirmer's values below the 10-mm cut off. In the group with reduced LG volumes, the Schirmer's scores were highly variable ranging from 3 to 35 mm. All the patients with normal LG presented normal Schirmer's values. A Schirmer's score less than 10 mm had a sensitivity of 68.4% to identify an LG volume of less than 0.20 cm^3 (Fig 1). Finally, the Fisher exact test revealed that the presence of SPK was significantly associated with an absent LG ($P = 0.0007$).

Orbital CT scans disclosed LG agenesis in 11 patients, 9 (42.8%) bilaterally and 2 unilaterally. Figure 2 shows examples of the morphologic LG variants detected. With the exception of 3 patients (6 glands), all LG volumes were reduced and below the minimum value measured in the control group (Table 2). In regard to only patients with BPES with measurable LG (Fig 3), median LG volumes were 0.22 cm^3 (range, 0.06−0.36 cm^3) and 0.24 cm^3 (range, 0.08−0.34 cm^3) concerning the right and left sides, respectively, with no significant difference between sides ($P = 0.72$). In the control group, median LG volumes were 0.54 cm^3 in the right orbits (mean 0.55 cm^3; standard deviation, 0.19; range, 0.30−1.22) and 0.54 cm^3 in the left orbits (mean, 0.53; standard deviation, 0.20; range, 0.32−1.29), values similar to those previously reported in the literature.[10] The Mann−Whitney test showed that the volumes of patients with BPES were significantly lower than in the control ($P < 0.05$) for both the right and left orbits.

Molecular Investigation

Molecular screening of the *FOXL2* gene showed pathogenic sequence variants in 13 (81.2%) of the 16 patients analyzed (Fig 4;