Clinical, Radiologic, and Genetic Features in Blepharophimosis, Ptosis, and Epicanthus Inversus Syndrome in the Indian Population

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Purpose. To study the clinical, radiologic, and genetic features in Indian Blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES) patients.

Methods. A total of 33 clinically well characterized BPES cases who presented between 2009 to 2011 were recruited. Clinical evaluation consisted of ophthalmic and orthoptic examination. For orbital indices, computed tomography (CT) scan of orbits was performed. Genetic studies included cytogenetic analysis and molecular analysis of FOXL2 gene.

Results. Significant clinical findings included a high incidence of refractive error in 94%, amblyopia in 60%, and strabismus in 40% of BPES cases. Orbital radiologic indices on CT scan in BPES were found to be comparable to the control group. On karyotyping, 8 out of 33 (24%) cases harbored chromosomal abnormalities. These abnormalities included 46,XY;del(3qter), 46,XX;del(3q26-25), and 46,XY;del(3q26qter). On molecular analysis, a novel mutation consisting of heterozygous substitution at c1635 that replaced cytosine by thymidine was detected.

Conclusions. To the best of our knowledge, this is the first study on clinical features in BPES patients of Indian origin. A high incidence of refractive error, strabismus, and amblyopia was found in BPES cases. Orbital imaging confirmed that clinical features are limited to soft tissue abnormalities, with no underlying bony changes. Cytogenetic studies showed that most chromosomal abnormalities in the Indian population are in the region of the long arm of chromosome 3. Results of molecular analysis indicate that there may be loci other than the FOXL2 gene, which are affected in BPES cases. Our study expands the existing mutation spectrum of FOXL2 gene.

Keywords: BPES, Indian, clinical features, genetics, FOXL2 gene
BPES is a rare disease and, as far as we are aware, clinical features in BPES cases of Indian origin have not been reported. Although generally believed to be a soft tissue disorder, radiologic biometric indices of the orbit in BPES patients have not been previously evaluated. Also, there have been very few genetic studies so far on the pattern of genetic abnormalities found in BPES cases in the Indian population.\textsuperscript{15-17} Therefore, we undertook a prospective study at our center to evaluate BPES patients with respect to clinical, genetic, and imaging findings.

**MATERIALS AND METHODS**

Patients with a clinical diagnosis of BPES who presented to the Oculoplastics service of Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi between 2009 and 2011 were recruited into the study, after obtaining informed consent. The study followed the tenets of the Declaration of Helsinki and prior approval from the ethics committee of All India Institute of Medical Sciences was obtained. For the clinical study, the control group consisted of age-matched individuals from a normal population. For radiologic study, the control group consisted of age-matched individuals who were referred to us for head and orbital computed tomography (CT) scan for other indications, in which the bony orbit was within normal limits. Clinical evaluation consisted of detailed ocular examination, which included assessment of visual acuity, refraction under cyclopia, measurement of horizontal and vertical palpebral apertures, levator function, anterior segment, and fundus evaluation. Orthoptic evaluation consisted of assessment of ocular alignment, ocular movements, and presence of amblyopia. Amblyopia was defined as Snellen visual acuity of less than 20/40, or an interocular difference in visual acuity of more than 1 Snellen line at the time of presentation.

Orbital imaging was performed using noncontrast Computed Tomography of the head and orbit in the department of Radiodiagnosis of our Institute. In all cases, 0.6- to 2-mm sections were obtained parallel to the infraorbital mental plane.\textsuperscript{18} Orbital biometry indices (Fig. 1) were studied in cases and controls.

For cytogenetic studies, chromosome analysis was done to identify any numerical or structural chromosomal aberrations.\textsuperscript{19,20} Lymphocyte cultures were set up and chromosomes were analyzed by G-banding. G-Banded metaphase images were captured with a Zeiss microscope (BX-51 Microscope; Olympus, Tokyo, Japan) and were analyzed using Cytovision 3.7 software (Applied Imaging Corp., Santa Clara, CA). At least 50 metaphases in each patient were analyzed and karyotyped.

DNA isolation, PCR amplification, and sequence analysis was performed. Genomic DNA was extracted from whole blood samples using the organic method described by Sambrook et al. with some modifications.\textsuperscript{21} The exon-intron regions of the FOXL2 were amplified in BPES patients. PCR amplifications for all primer sets (Table 1) were performed in a 25-µL volume containing 1.0 µL of 20 mM stock solution for each primer (Eurofins Genomics India Pvt Ltd., Bangalore, India), 100 ng of genomic DNA, 1 unit of Taq polymerase (Bangalore Genei, Bengaluru, Karnataka, India), 0.1 mM deoxynucleotide triphosphate (dNTP), and 4 µL of 10X PCR buffer (with 15 mM MgCl\textsubscript{2}). Amplified PCR products were purified using a gel/PCR DNA fragments extraction kit (Geneaid Biotech Ltd., Sijih City, Taiwan). Purified PCR products were sent for sequencing to Molecular Cloning Laboratories (South San Francisco, CA). All fragments were sequenced in both forward and reverse directions for confirmation of any nucleotide variation in cases and controls and compared with the Human Genome Reference Sequence (NC_000003.11) provided by the National Center for Biotechnology Information (NCBI), using ClustalW2 (multiple sequence alignment program for DNA; European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK).

Computational assessment of missense mutations was done. Two homology based programs, Polymorphism Phenotyping (PolyPhen), available in the public domain at http://genetics.bwh.harvard.edu/pph/, and Sorting Intolerant From Tolerant (SIFT) analysis tool, available in the public domain at http://sift.jcvi.org/, were used to predict the functional impact of missense changes identified in this study. PolyPhen structurally analyzes an amino acid polymorphism and predicts whether that amino acid change is likely to be deleterious to protein function.\textsuperscript{22,23} PolyPhen scores of greater than 2.0 indicate the polymorphism is probably damaging to protein function. Scores of 1.5 to 2.0 are possibly damaging, and scores of less than 1.5 are likely benign. SIFT is based on the premise that protein evolution is correlated with protein function. Positions with normalized probabilities less than 0.05 are predicted to be deleterious and those greater than or equal to 0.05 are predicted to be tolerated in case of SIFT.

The main outcome measures of our study were comparison of clinical parameters and radiologic biometric measurements between BPES cases and age-matched controls, and results of cytogenetic and molecular analysis. Appropriate statistical analysis was performed using SPSS (version 14.0; SPSS Inc., Chicago, IL). Quantitative data was classified as parametric and nonparametric data. Student’s t-test was used for parametric and Mann-Whitney U test was used for nonparametric data. χ\textsuperscript{2} test or Fischer test was used for qualitative data. A P value less than or equal to 0.05 was considered significant.

**RESULTS**

A total of 33 clinically well characterized BPES cases were enrolled in the study.

![CT scans of orbits](image-url)
Clinical Results

The mean age in BPES cases was 12 ± 8.4 years (range, 4–32 years), with the majority of cases between 4 to 8 years. There were 16 (48%) males and 17 (52%) females. All females were in the prepubertal age group. Of 33 patients, 31 cases (94%) had bilateral ptosis and two cases (6%) had unilateral ptosis. Ptosis was severe in 28 cases (85%) and moderate in five cases (15%). Associated lid anomalies were noted in five patients (15%). These included lower lid entropion on the medial side in four cases, and a lower lid ectropion in one case (Fig. 2).

Refractive errors were detected in 31 patients (94%). The most common refractive errors included simple hyperopia in 12 cases (36%), and a with-the-rule astigmatism in 12 cases (36%). The incidence of amblyopia in BPES was 60% (n = 20). Of these, 15 cases (45%) had bilateral amblyopia and 5 (15%) cases had unilateral amblyopia. Strabismus was noted in 13 cases (40%). Of these, 10 cases (30%) had esotropia and 3 cases (10%) had exotropia.

Other clinical parameters that were evaluated are summarized in Table 2. A statistically significant difference was noted between cases and controls in the mean value of horizontal palpebral aperture (P = 0.0001), vertical palpebral aperture (P = 0.0001), and intercanthal distance (P = 0.0001). There were no statistically significant differences between cases and controls in the mean values of keratometry, corneal thickness, and intraocular pressure (IOP) (Table 2).

Radiologic Results

The results of orbital biometric indices as determined on CT scan are summarized in Table 3. There was no statistically significant difference between cases and controls in the mean values of interpupillary distance and interorbital distance. Similarly, other indices such as zygomatic–zygomatic bone distance, optic nerve–optic nerve distance, optic canal–optic canal distance, and optic nerve–optic canal distance were also found to be comparable between cases and controls.

Genetic Results

A total of 33 BPES cases (27 proband and 6 family members) and age-matched controls underwent cytogenetic analysis to detect any chromosomal abnormality. Of these, 24 cases were sporadic and three probands had a positive family history. These three patients belonged to different families, with two affected members apart from proband in each family. There was no history of consanguinity. Eight out of 33 cases (24%) harbored chromosomal abnormalities on cytogenetic analysis. Among cytogenetically positive cases, three cases were sporadic and five were familial. All deletions were found on the long arm of chromosome 3. Four patterns of deletions were observed (Table 4, Fig. 3). The first pattern showed a deletion in the terminal region of the long arm of chromosome 3 (46, XYdel 3qter). This pattern was seen in a sporadic case. The second pattern had a deletion on the long arm of chromosome 3 in region 26.3 (46, XYdel 3q26.3). This pattern was seen in two familial cases, with affected father and daughter. The third pattern had a deletion on the long arm of chromosome 3 in the region 24-25 (46, XXdel 3q24-25). This pattern was seen in one...

### Table 1. Primer Sequence for Exon of BPES Gene

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Primer Sequence</th>
<th>Annealing Temp, °C</th>
<th>Product Size, bp</th>
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</thead>
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<td>FOXL2_1_AF</td>
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<td>57</td>
<td>893</td>
</tr>
<tr>
<td>FOXL2_1_AR</td>
<td>5’AGTATGAGTCCTGTTGCTGCGC3’</td>
<td>59</td>
<td>899</td>
</tr>
<tr>
<td>FOXL2_1_BF</td>
<td>5’CGAAGTCGCTGTTGCTGCGC3’</td>
<td>58</td>
<td>863</td>
</tr>
<tr>
<td>FOXL2_1_BR</td>
<td>5’GCTGTCGCTGTTGCTGCGC3’</td>
<td>57</td>
<td>945</td>
</tr>
<tr>
<td>FOXL2_1_CF</td>
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<tr>
<td>FOXL2_1_DR</td>
<td>5’GCTGTCGCTGTTGCTGCGC3’</td>
<td>57</td>
<td>945</td>
</tr>
</tbody>
</table>

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**Figure 2.** Clinical photograph of a patient with BPES showing lower lid ectropion in the left eye, with asymmetric ptosis.

**Figure 3.** G-banded images showing different abnormalities in chromosome 3. (A) 46,XYdel (3q 26.3), (B) 46,XXdel(3q24-25), (C) 46,XYdel(3q26-qter), and (D) 46,XYdel(3qter).
sporadic and three familial cases with affected father, son, and daughter. The fourth pattern also had a deletion on the long arm of chromosome 3 near the terminal region (46, Xqydel 3q26–qter). This pattern was seen in a sporadic case.

Patients who were cytogenetically normal (n = 25) underwent molecular analysis for detection of FOXL2 sequence variations. Of these, one patient with a positive family history and affected sibling showed a mutation located in the 3′ UTR region of the FOXL2 gene. This change was heterozygous c1635 cytosine > thymidine substitution that replaced cytosine by thymidine in the affected individual (Fig. 4).

**DISCUSSION**

BPES is a complex eyelid malformation characterized by four cardinal ocular features, which include blepharophimosis, ptosis, epicanthus inversus, and telecanthus. This prospective study was undertaken to evaluate the clinical features, radiologic findings, and genetic profile of FO XL2 patients in the Indian population.

The most common age group at the time of diagnosis in the current study was 4 to 8 years. This finding is consistent with studies from other parts of the world that have reported that the majority of cases present before 8 years of age. In our series, 52% of affected individuals were females and 48% were males. Previous investigators have reported an incidence of 30% to 54% in males and 46% to 70% in females, and our results are within this range. In our patients, a positive family history was noted in 9 out of 33 (27%) cases.

All BPES cases in our study had ptosis, which included severe ptosis in 85% and moderate ptosis in 15% cases. In other studies, severe ptosis has been reported in 60% to 70% cases and moderate ptosis in 30% to 40% cases. The incidence of strabismus in BPES cases has been reported as 20% to 55%, which included esotropia in 30% and exotropia in 10% cases. Although previously described in literature, none of our cases had vertical deviations. The incidence of refractive error was 95% in our series, which is significantly higher than the previously reported range of 54% to 70%. We found simple hyperopia and astigmatism to be the most common types of refractive error in our patients.

Amblyopia screening forms an important part of evaluation in BPES cases because of its high incidence. Previous investigators have reported the incidence of amblyopia in BPES from 39% to 41%. Our results show a higher incidence of amblyopia (60%). This could be attributed to a lack of awareness in the general population about the importance of early screening. Interestingly, the incidence of amblyopia was 70% in the strabismus group as compared with 40% in the nonstrabismus group, although the difference was not statistically significant (P = 0.3). Previous studies have reported that strabismus is a cause of amblyopia in 46% to 67% of BPES cases. Severe ptosis is also a known risk factor for the development of amblyopia and we found a higher incidence of amblyopia (70%) in cases with severe ptosis.

<table>
<thead>
<tr>
<th>TABLE 2. Clinical Parameters in BPES Cases and Controls</th>
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<tbody>
<tr>
<td><strong>Parameters</strong></td>
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<td>----------------</td>
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<tr>
<td>Horizontal palpebral aperture OD, mm</td>
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<tr>
<td>Horizontal palpebral aperture OS, mm</td>
</tr>
<tr>
<td>Vertical palpebral aperture OD, mm</td>
</tr>
<tr>
<td>Vertical palpebral aperture OS, mm</td>
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<tr>
<td>Intercanthal distance, mm</td>
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<td>Keratometry 2 OD, D</td>
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<tr>
<td>Keratometry 1 OS, D</td>
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<tr>
<td>Keratometry 2 OS, D</td>
</tr>
<tr>
<td>Central corneal thickness OD, μm</td>
</tr>
<tr>
<td>Central corneal thickness OS, μm</td>
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<tr>
<td>IOP OD, mm Hg</td>
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<tr>
<td>IOP OS, mm Hg</td>
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</table>

**TABLE 3. Radiologic Parameters in BPES Cases and Controls**

<table>
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<th><strong>Controls, mm</strong></th>
<th><strong>P Value</strong></th>
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<tr>
<td>IPD</td>
<td>55.4 ± 4.4</td>
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<tr>
<td>IOD 1</td>
<td>23.3 ± 5.2</td>
<td>23.5 ± 2.4</td>
<td>0.47</td>
</tr>
<tr>
<td>IOD 2</td>
<td>24.7 ± 3.9</td>
<td>24.2 ± 2.9</td>
<td>0.35</td>
</tr>
<tr>
<td>ZOD</td>
<td>83.7 ± 7.6</td>
<td>82.7 ± 6.9</td>
<td>0.51</td>
</tr>
<tr>
<td>NN</td>
<td>46.2 ± 5.2</td>
<td>46.4 ± 2.9</td>
<td>0.57</td>
</tr>
<tr>
<td>CC</td>
<td>22.9 ± 1.5</td>
<td>23.0 ± 1.4</td>
<td>0.64</td>
</tr>
<tr>
<td>ON OD</td>
<td>25.5 ± 2.9</td>
<td>25.5 ± 2.8</td>
<td>0.49</td>
</tr>
<tr>
<td>ON OS</td>
<td>25.6 ± 2.9</td>
<td>25.5 ± 2.9</td>
<td>0.45</td>
</tr>
</tbody>
</table>

**FIGURE 4. FOXL2 Gene Analysis Chromatogram showing a portion of FOXL2 gene DNA sequence in an affected and an unaffected individual. (Top) DNA sequence chromatogram showing the heterozygous c1635 C > T substitution that replaced cytosine by thymidine in the affected individual. (Bottom) DNA sequence chromatogram of an unaffected individual showing wild type C at position c1635. The position of mutated (C > T) and wild type nucleotide C in an affected and an unaffected individual is indicated in the box.**
Furthermore, all cases with amblyopia in the current study had associated refractive error. These observations suggest that the development of amblyopia in BPES cases is multifactorial and could be attributed to all three factors (i.e., strabismus, refractive error, and severe ptosis).

Other clinical parameters that were evaluated included IOP and corneal curvature; we studied these parameters because BPES has been reported to be associated with ocular associations such as angle dysgenesis, elevated IOP, and irregular astigmatism (Sippel KC, et al. IOVS 2002;43:ARVO E-Abstract 178). None of these parameters showed any statistically significant difference as compared with controls. Although associated systemic abnormalities such as developmental delay, growth deficiency, and cleft palate have been reported in literature,25 none of our cases showed any obvious systemic or developmental abnormality.

Radiologic biometry was undertaken to study the bony development of the orbit in BPES cases. As far as we are aware, radiologic biometric indices of the orbit have not been previously reported in BPES. Although generally believed to be a soft tissue disorder, bony abnormality of the orbit have been described in a previous study on BPES patients.26 In a series of 50 BPES cases (which included 44 children),26 the authors performed CT scan of the orbits and observed that BPES may be associated with orbital phimosis (i.e., a deep and wide orbit).26 In such cases, they recommended orbital reconstructive surgery and bone remodeling to enlarge and widen the orbital framework and to shorten the orbital cavity.26 A limitation of their study was the absence of a control group to compare the radiologic findings. In our study, none of the parameters in BPES cases showed any statistically significant difference as compared with age-matched controls, thereby confirming that in BPES, it is only the soft tissues that are affected and bony development remains within normal limits. The lacrimal gland was normal in all our BPES cases, although a sporadic case report of BPES and absence of lacrimal gland has been reported in literature.27 Recently, magnetic resonance imaging (MRI) features of levator palpebrae superioris muscle (LPS) have been reported in a small cohort of BPES cases. In our patients, we performed CT scans as it is preferable to MRI to study the osseous details.28

Literature review of cytogenetic anomalies in BPES shows that the cytogenetic rearrangements in BPES mostly consist of interstitial deletions and/or translocations involving the long arm of chromosome 3, similar to those observed in our study.29–32 Our results are consistent with previously reported cytogenetic abnormalities in BPES.29–32 A previous study has shown linkage of blepharophimosis syndrome in a large Indian pedigree to chromosome 3p.33 Patients who had a negative result on cytogenetic analysis in our study underwent further molecular analysis for detection of the FOXL2 mutation. FOXL2 was chosen considering the fact that the maximum sequence variations in BPES have been found in this gene. Among all genetic defects found in BPES, an estimated 72% of cases are due to intragenic FOXL2 mutations54; 12% of BPES cases result from deletions involving partial or whole FOXL2 gene deletion,57 and approximately 5% of cases involve regulatory deletions outside the FOXL2 gene.55 In our study, of 25 cases, one proband and one affected family member showed a positive result. The mutation detected in this instance was a novel mutation on FOXL2 gene. The case was heterozygous for this mutation (c1635 cytosine > thymidine) and was located in the 3’ UTR region of the gene. To the best of our knowledge, 18 mutations have been found in the 3’ UTR region of FOXL2 gene, but the change reported in this study is novel.

Literature review shows that almost all intragenic mutations in FOXL2 gene identified until date are confined to the single coding exon of FOXL2, which is why it was also selected in our study. However, genetic defects in BPES may involve other loci, and one limitation of our study is that we confined molecular analysis only to FOXL2 gene. The low prevalence of FOXL2 mutation in our patient population could be partly attributed to a different ethnic population and very few cases with a positive family history. Previous genetic studies on Indian BPES patients have been reported from the southern parts of India,15–17 where the population is ethnically different from Northern India and a high number of consanguineous marriages are found, resulting in a different mutation spectrum. In our study, seven out of nine familial cases (77%) were found to be positive for genetic defects, which is consistent with western studies in which 72% of clinically diagnosed BPES cases have a mutation in the FOXL2 gene. However, the rate of mutation in sporadic cases in our cohort was only 12.5%, indicating that there may be loci other than the FOXL2 gene, which are affected in BPES patients from Northern India. Further studies to explore other possible loci which may regulate the development of eyelids in BPES would be required.

To summarize, this is a comprehensive study on BPES cases which reports the clinical, radiologic, and genetic findings in BPES cases in the Indian population. BPES was found to be associated with a high incidence of refractive error, strabismus, and amblyopia. Orbital radiologic indices in BPES cases were comparable with the normal population, thereby confirming that the disease is related only to soft tissue developmental abnormality and bony development is essentially normal. This study showed that most chromosomal abnormalities in the Indian population are in the region of the long arm of chromosome 3. While our study expands the existing mutation spectrum of FOXL2 gene, there may be loci other than the FOXL2 gene that are involved in BPES cases.
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