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## RESEARCH NOTE

## FOXL2 mutations in Indian families with blepharophimosis–ptosis–epicanthus inversus syndrome

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### Introduction

Blepharophimosis–ptosis–epicanthus inversus syndrome (BPES) (MIM#110100) is an autosomal dominant genetic condition characterized by a complex eyelid malformation. The main features of BPES are shortening of the horizontal palpebral fissures (blepharophimosis), congenital ptosis, telecanthus and epicanthus inversus, and variable expressivity of female infertility (premature ovarian failure) (POF) in type I BPES while absent in type II (Zlotogora *et al.* 1983).

Both types of BPES are caused by mutations in the *FOXL2* gene (MIM# 605597), consisting of a single exon of 2.7 kb located on chromosome 3q23 (Crisponi *et al.* 2001). It encodes a protein that belongs to the family of winged-helix/forkhead transcription factors that contains 100 amino acid DNA-binding forkhead domain and a polyalanine tract. *FOXL2* expression is restricted to developing eyelids of fetal and adult ovarian granulosa cells (Cocquet *et al.* 2003).

In previous mutation studies intragenic mutations were found in 70% of patients (De Baere *et al.* 2003). Recently, in sporadic and familial BPES cases five microdeletions were found outside the coding region of *FOXL2*. In addition, 11 rearrangements encompassing *FOXL2* have been described. Overall, genomic rearrangements encompassing or outside the *FOXL2* account for 16% of all molecular defects found in BPES families (Beysen *et al.* 2005).

Genotype–phenotype correlations suggested that *FOXL2* mutations resulting in predicted proteins with a truncation

before the polyalanine tract might lead to development of the POF (BPES type I). However, correlations for mutations leading to a truncated or extended protein containing an intact forkhead domain and polyalanine tract, and for missense mutations in the highly conserved forkhead domain, are still elusive. Large submicroscopic deletions may result in BPES associated with mental retardation (De Baere *et al.* 2003; Beysen *et al.* 2005).

Here we report the identification of intragenic *FOXL2* mutations in two Indian families with BPES type I and II respectively. We demonstrate that our results are in agreement with previously reported genotype–phenotype correlations.

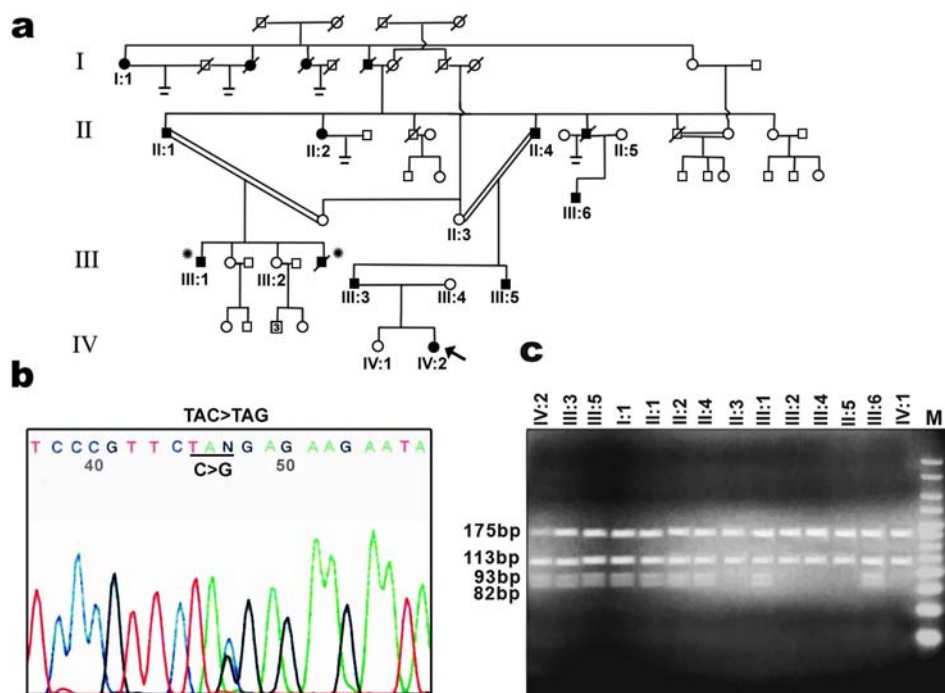
### Materials and methods

#### Patients and clinical evaluation

Recruitment was based on interviews, questionnaires, and clinical examination of affected and unaffected individuals by ophthalmologists. An informed consent was obtained in compliance with the Helsinki Declaration. Clinical data are shown in table 1. The pedigrees of the two families are shown in figure 1a and figure 2a. A four-generation BPES type I family with 14 affected members of which nine are alive, and were selected for genetic analysis (figure 1a). The proband was a one-year-old girl with typical BPES and not cooperative for visual acuity testing. However, adapted visual acuity testing revealed an acuity of at least 6/24 or better. Cycloplegic refraction revealed +0.5 D for the right eye and +1.0 D for the left eye.

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**Keywords.** BPES syndrome; *FOXL2* gene; haploinsufficiency; premature ovarian failure (POF); *FOXL2* polyalanine expansion; genetic counseling.



**Figure 1. a.** Four-generation BPES type I pedigree. All affected members have typical BPES. Three affected females from generations I and II are infertile. Affected individual BPES III:1 has mental retardation and microcephaly. The affected and unaffected patients analysed molecularly are indicated by numbers “IV:1, III:3, etc”. An asterisk indicates the two affected individuals having mental retardation and microcephaly. **b.** Sequence electropherogram showing the heterozygous *FOXL2* mutation c.273C > G (p.Y91X) found in affected members of this BPES type I family. **c.** Segregation analysis in affected and unaffected family members using *Bse*LI restriction enzyme digestion. The *Bse*LI restriction enzyme showed 175 bp and 113 bp in the wild type allele while additional fragments of 82 bp and 93 bp were observed in mutant alleles. These fragments were separated on a 2.5% agarose gel.

Another three-generation BPES type II family had three affected members, with a three-year-old male proband with a typical BPES (figure 2a). Visual acuity recorded by Cardiff acuity chart and Teller preferential looking charts was 6/9 in both eyes. The cycloplegic refraction was +1.0 D in both eyes. The probands of both the families underwent frontalis sling surgery in both the eyes for ptosis and Y-V plasty for telecanthus correction.

#### Mutation analysis

Blood samples were collected from consenting individuals followed by extraction of genomic DNA following a standard protocol. The coding region of the *FOXL2* gene was PCR amplified using previously reported primers (De Baere et al. 2003; Kumar et al. 2004). The PCR amplicons were sequenced bidirectionally (3730 ABIPRISM Analyzer, Big Dye Terminator, Applied Biosystems) followed by RFLP analysis. The segregation of the mutation in the BPES type I family was assessed by restriction enzyme digestion using 2 units of *Bse*LI (MBI FERMANTAS) followed by electrophoresis on a 2.5% agarose gel (figure 1c).

#### Results and discussion

In a four-generation BPES type I family with nine living affected members, three affected females are infertile and two affected male patients (BPES III:1 and his younger brother) have mental retardation with microcephaly (figure 1a). In all the affected individuals analysed a nonsense mutation c.273C > G (p.Y91X) was identified in *FOXL2*, leading to a predicted truncated protein of 90 amino acids (instead of 376) with a partial forkhead domain and polyalanine tract is absent (figure 1b,c).

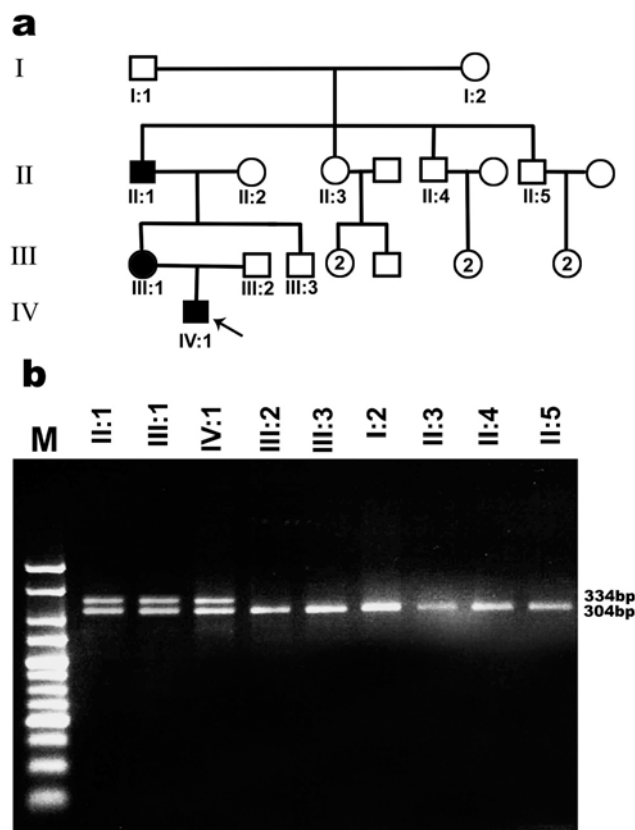
These mutations are supposed to lead to haploinsufficiency of *FOXL2*, since DNA-binding activity is abolished due to disruption of the forkhead domain. Although in general, transcripts with nonsense mutation are degraded by nonsense-mediated decay (NMD), intronless genes as the *FOXL2* gene might escape NMD, giving rise to a stable mutated transcripts and expressed mutated proteins (Frischmeyer and Dietz 1999).

In general, mutations leading to the predicted proteins with a partial forkhead domain are expected to lead to BPES type I as suggested previously (De Baere et al. 2003). Indeed this nonsense mutation leads to BPES type I in this family.

**Table 1.** Clinical features of Indian families with blepharophimosis-ptosis-epicanthus inversus syndrome.

BPES families	Affected individuals	Age/Sex	BPES	ICD (mm)	OCD (mm)	PFL (mm)	Mental retardation	Microcephaly	Infertility	Consanguinity
Four generation Type I (figure 1)	IV: 2	1/F	+	33	74	3.5	-	-	Unknown	-
	III: 2	32/M	+	35	88	3	-	-	-	+
	III: 5	27/M	+	NM	NM	NM	-	-	-	+
	I: 1	90/F	+	36	92	4	-	-	+	-
	II: 1	68/M	+	NM	NM	NM	-	-	-	-
Three generation Type II (figure 2)	II: 2	65/F	+	NM	NM	NM	-	-	+	-
	II: 4	62/M	+	38	90	2	-	-	-	-
	III: 1	40/M	+	NM	NM	NM	+	+	-	+
	III: 6	10/M	+	NM	NM	NM	-	-	-	-
	II: 1	53/M	+	33	90	5.5	-	-	-	-
	III: 1	27/F	+	30	94	6.5	-	-	-	-
	IV: 1	3/M	+	33	78	6	-	-	-	-
	III: 2									
	III: 3									
	II: 5									

ICD, inner canthal distance; OCD, outer canthal distance; PFL, palpebral fissure length; BE, right eye, LE, left eye; NM; not measured “+”, Present; “-”, absent.



**Figure 2.** a. Three-generation BPES type II pedigree. The affected and unaffected patients analysed at the molecular level are indicated by numbers “II:1, III:1, etc”. b. Segregation analysis of the 30-bp *FOXL2* duplication c.672\_701dup “g.909\_938” using gel electrophoresis on a 2.5% agarose gel, in affected (heterozygous PCR fragment of 304 bp and 334 bp) and unaffected family members (homozygous fragment of 304 bp).

Interestingly, two affected brothers have BPES with mental retardation and microcephaly (figure 1a and table 1). The younger brother died and could not be clinically examined to undergo molecular testing; the older one however (BPES III:1) was shown to carry the familial *FOXL2* mutation. It is not clear whether these associated features are caused by the familial *FOXL2* mutation in these brothers. Intragenic *FOXL2* mutations are known to occur in BPES individuals with normal psychomotor development and normal head circumference (De Baere *et al.* 2003). It is important to mention that these mentally retarded brothers descend from a consanguineous marriage, their parents being first cousins. Thus it cannot be excluded that the mental retardation and microcephaly result from a different recessive condition independent from BPES. Notably, one of the affected females (proband BPES IV:2) is a one-year-old female of whom the BPES type cannot be assessed due to her prepubertal age (table 1). However, she is predicted to develop BPES type I, which has implications for genetic counseling.

In addition we identified a recurrent 30-bp duplication

c.672\_701dup “g.909\_938” in the *FOXL2* gene in a three-generation family affected by BPES type II (figure 2a). This duplication cosegregated with the disease in this family (figure 2b). This mutation leads to an inframe polyalanine expansion in the FOXL2 protein (p.A224\_A234 dup). It has been reported as a pathogenic change in sporadic and familial cases in other ethnicities, but this is the first report in an Indian pedigree with BPES (Human *FOXL2* Mutation Database; <http://mebgen.ugenp.be/foxl2>).

This polyalanine expansion represents more than 30% of intragenic *FOXL2* mutations. It is predicted to result in a hypomorphic allele and lead to BPES type II. Recently, it was proposed that the pathogenic mechanism of this polyalanine expansion may be its mislocalisation (due to cytoplasmic aggregation) together with its inclusion into nuclear aggregates (Caburet et al. 2004). We have recently identified a short homozygous polyalanine expansion (of 19 alanines instead of 14) in a consanguineous Indian pedigree, resulting in a recessive form of BPES and ovarian dysfunction (Nallathambi et al. 2007). The findings in our family lend further support to the genotype–phenotype correlation for this type of mutation (polyalanine expansion).

In summary, we have reported a nonsense mutation in a BPES type I family and a recurrent duplication leading to a polyalanine expansion in a BPES type II family, both are of Indian origin. This is the first study where genotype–phenotype correlations have been possible in Indian BPES families. They are in agreement with previous mutation studies in BPES. Genotype–phenotype correlations in BPES are especially useful for predictive testing of POF risk in affected females.

#### Acknowledgements

The authors are grateful to the participants of this study. Authors thank Dr V. R. Muthukkaruppan, Director of Research, Aravind

Medical Research Foundation for his valuable suggestions. We also thank V. R. Muthulakshmi and B. Suganthi for sample collection, T. P. Vasanthi, for technical assistance and Mr R. Jeyakrishnan & Mr. M. Rajkumar for photographic work.

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Received 27 January 2006