

Title: Blepharimosis, Ptosis, and Epicanthus Inversus *GeneReview* – Observed phenotypic spectrum associated with *FOXL2* pathogenic variants

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## Intragenic Mutations

For some *FOXL2* pathogenic variants, inter- and intrafamilial variable expressivity of the ovarian phenotype (female infertility, premature ovarian insufficiency) is observed [De Baere et al 2003].

**Pathogenic variants predicted to result in proteins truncated before the polyalanine tract** preferentially lead to POI (BPES type I). Note: The need for careful interpretation of genotype-phenotype correlations is illustrated by the co-occurrence of BPES type I and isolated POI in a three-generation family in which all individuals with BPES had the nonsense mutation c.244C>T (p.Gln82Ter) and the females with isolated POF did not. Although this mutation belongs to a group of mutations that is preferentially associated with BPES type I [De Baere et al 2003], it is unclear whether the POI in the women in this family results from the *FOXL2* pathogenic variant or from another genetic or non-genetic cause [Beysen et al 2008, Beysen et al 2009].

**Polyalanine expansions** preferentially lead to BPES type II. The first case with a positive correlation between the size of the polyalanine expansion, its dosage, and the penetrance of the BPES phenotype was reported by Nallathambi et al [2007]. In a consanguineous Indian family, individuals heterozygous for a short polyalanine expansion of 19 alanines (c.684\_698dup15; p.Ala230\_Ala234dup) were unaffected, but individuals who were homozygous had typical BPES (with documented POI in one female) [Nallathambi et al 2007]. This was the first report on a homozygous *FOXL2* pathogenic variant providing evidence of a recessive form of BPES associated with ovarian dysfunction. Note: The polyalanine expansion does not result from a simple trinucleotide repeat (see Table 3, footnote 1).

- Variable degrees of ovarian dysfunction were observed in seven women with BPES who were heterozygous for a *FOXL2* allele with a poly-Ala expansion. However, when hormonal status could be assessed, hypergonadotropic hypogonadism was not observed, suggesting that these polyalanine expansion mutations may result in late-onset ovarian insufficiency [Beysen et al 2009].
- A 16-year old young woman thought to have BPES type I with the poly-Ala expansion c.667\_702dup (p.Ala221\_Ala234dup) had an extremely large corpus luteum cyst that caused transient ovarian dysfunction [Raile et al 2005]. Although it was postulated that this transient ovarian insufficiency might be caused by malfunction of the *FOXL2* protein, the ovarian dysfunction seen in BPES type I is

progressive, not transient. However, the role of the compressing cyst or cystectomy in the cause of ovarian dysfunction in this patient is unclear.

**Mutations that predict a truncated or extended protein** containing an intact forkhead and polyalanine tract are not known to have a genotype-phenotype correlation.

**Missense mutations in the forkhead domain**, in general, do not correlate with ovarian phenotype. However, recent studies might offer some predictive value regarding ovarian phenotype.

- Missense mutations in the forkhead domain that lead to mislocalization and aggregation (and, thus, severely impair transactivation) tend to have a more severe ovarian phenotype than missense mutations that do not significantly affect protein localization and function [Beysen et al 2008].
- Di Pietromaria et al [2009] developed a prediction tool for *FOXL2* intragenic (missense and other) mutations, the validation of which was based on known phenotypic effects of a 'training set' of pathogenic variants (BPES type I or type II). A clear correlation was found between the transcriptional activity of *FOXL2* pathogenic variants on two different reporter promoters and the BPES type.
- In a recent study by Todeschini et al [2011], the amino acids of the helices of the forkhead domain of *FOXL2* were systematically replaced by glycine residues to assess the impact of these artificial mutations. A number of pathogenic variants led to protein mislocalization, aggregation and to partial or complete loss of transactivation ability on a dozen of luciferase reporter systems. No clear-cut correlation was found between protein mislocalization or aggregation and the position of the pathogenic variant. However, the localization of the side chain of each amino acid was found to correlate very well with the impact of its mutation on *FOXL2* transactivation capacity. Extrapolation of this analysis to natural mutations was in agreement with the findings obtained for the artificial mutations. This study brought important insights into the molecular effects of *FOXL2* missense mutations located in the forkhead domain, and provided an apparently reliable in silico predictive tool for their phenotypic effects [Todeschini et al 2011].
- Fan et al [2011] performed computational analysis of a novel missense mutation in a three-dimensional structural model and hypothesized that the pathogenic variant might disturb the intermolecular contacts between *FOXL2* and *STAR* (*StAR*). The disturbance of this interaction might contribute to the POI observed in women with BPES type I. In addition, they performed subcellular localization and functional studies, and observed significant nuclear aggregation and cytoplasmic mislocalization of the mutant type protein. Loss-of-function was confirmed by electrophoretic mobility shift assays, transcriptional activity assays and quantitative real-time polymerase chain reaction.

**Missense mutations outside the forkhead domain.** Three pathogenic variants downstream of the forkhead domain (p.Tyr215Cys, p.Ser217Phe and p.Ser217Cys) had a mild BPES phenotype [Kumar et al 2004, Beysen et al 2008, Nallathambi et al 2008]. Recently, the p.Ser217Cys pathogenic variant was identified in a family with a severe BPES phenotype [Haghighi et al 2012].

**Additional findings observed with some intragenic mutations.** Although intragenic *FOXL2* mutations usually lead to BPES type I or II without any associated findings, the following case reports describe individuals who have additional atypical features that could result from pleiotropic effects of these pathogenic variants.

- A ventricular septal defect (VSD) was found in an individual with a poly-Ala expansion (c.672\_701dup; p.Ala225\_Ala234dup) and one with a missense mutation in the forkhead domain (c.205G>A; p.Glu69Lys).
- Developmental delay was reported in: two affected males of a four-generation family with BPES type I (c.273C>G; p.Tyr91Ter); a four-year-old simplex case (i.e., a single occurrence in a family) (c.663\_692dup; p.Ala225\_Ala234dup); and an 11-year old girl who was a simplex case (c.1056delG; p.Glu352AspfsTer4).
- The combination of a complex heart defect and severe developmental delay was described in a one-year-old simplex case with the pathogenic variant c.665C>T (p.Gln219Ter).
- An association between BPES and Duane syndrome was found in a one-year-old with an expansion of the poly-Ala tract (c.672\_701dup; p.Ala225\_Ala234dup) [Vincent et al 2005]. The same pathogenic variant was found in a 12-year old male who had had Burkitt lymphoma.
- In another family with the c.663\_692dup (p.Ala221\_Ala231dup) pathogenic variant, a seven-year old male had BPES and a cleft palate (Pierre Robin sequence) and his mother had typical BPES. An individual with the missense mutation c.305T>C (p.Ile102Thr) had a cleft lip.
- Growth hormone deficiency, which has previously been described in two individuals with BPES without any other associated findings [Wales 1998, Varghese et al 2002], was found in one individual with the 17-bp duplication c.672\_701dup (p.Ala225\_Ala234dup) [Crisponi et al 2002] and two sisters with the missense mutation c.650C>T (p.Ser217Phe). In one individual with BPES with the pathogenic variant c.500\_501delTCinsAA [Crisponi et al 2001], growth retardation was observed but growth hormone was not assayed.

Growth hormone deficiency may be attributed to *FOXL2* expression in Rathke's pouch [Treier et al 1998]. *FOXL2* is essential for pituitary development and function and *FOXL2* expression precedes expression of genes involved in gonadotrope-specific development [Ellsworth et al 2006]. However, most individuals with BPES do not have recognizable pituitary abnormalities, suggesting that the pituitary is less sensitive to *FOXL2* dosage than the developing eyelids and ovary.

It is rather unlikely that the other associated features mentioned (e.g., growth hormone deficiency, Duane syndrome) result from a wider pleiotropic effect of *FOXL2* in development.

## Genomic Rearrangements

**Deletions encompassing *FOXL2*.** No reliable genotype-phenotype correlations with respect to POF could be established [Beysen et al 2005, D'haene et al 2010].

Although it was postulated that intellectual disability in individuals with a microdeletion of the *FOXL2* region could be attributed to haploinsufficiency of ATR [de Ru et al 2005], a consistent correlation could not be found [Beysen et al 2005].

In a larger cohort of deletions encompassing *FOXL2*, all individuals with deletions located entirely within the interval chr3:139780672-142467395 (~2.7 Mb, hg18) showed normal psychomotor development. The identification and sizing of a *FOXL2*-encompassing deletion in a newborn with BPES can give a prognostic indication for psychomotor development [D'haene et al 2010]. Another preliminary correlation was suggested by Wilmore and colleagues, as they hypothesized that *SOX14*, located in the vicinity of *FOXL2*, is a candidate gene for the limb anomalies which are occasionally observed in persons with BPES that include brachydactyly, syndactyly, camptodactyly, clinodactyly, joint abnormalities, and club foot [Wilmore et al 2000 and references therein].

Athappillya et al [2009] reported an individual with BPES and congenital alacrima caused by interstitial deletion of the long arm of chromosome 3 [46, XX, del(3)(q22.2q23)].

Zahanova et al [2012] reported an individual with characteristic facial features of BPES and with genital anomalies, spastic diplegia, and speech delay caused by deletion of 3q22.3q23.

**Deletions outside *FOXL2*.** The BPES type could only be assessed in two of nine families, which appeared to have BPES type II [Beysen et al 2005, D'haene et al 2009]. Developmental delay was not observed.

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