GENEReviews

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Blepharophimosis, Ptosis, and Epicanthus Inversus

[*BPES, Blepharophimosis Syndrome. Includes: Blepharophimosis, Ptosis, and Epicanthus Inversus Type I (BPES I); Blepharophimosis, Ptosis, and Epicanthus Inversus Type II (BPES II)*]

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Summary

Disease characteristics. Classic blepharophimosis syndrome (BPES) is a complex eyelid malformation invariably characterized by four major features: blepharophimosis, ptosis, epicanthus inversus, and telecanthus. Two types of blepharophimosis syndrome have been described: BPES type I includes the four major features and female infertility caused by premature ovarian failure (POF); BPES type II includes only the four major features. Other ophthalmic manifestations associated with BPES include lacrimal duct anomalies, amblyopia, strabismus, and refractive errors. Minor features include a broad nasal bridge, low-set ears, and a short philtrum. Individuals with BPES and an intragenic disease-causing mutation are expected to have normal intelligence.

Diagnosis/testing. The diagnosis of BPES is primarily based on clinical findings. Occasionally individuals with BPES have cytogenetic rearrangements, such as interstitial deletions and translocations involving 3q23. *FOXL2* is the only gene currently known to be associated with BPES. Mutations are identified in approximately 80% of affected individuals by using a combination of sequence analysis of the coding region (single exon) and deletion testing using methods such as multiplex ligation-dependent probe amplification (MPLA) and fluorescent in situ hybridization (FISH). Such testing is clinically available.

Management. Timing of eyelid surgery involves weighing the balance of early surgery to prevent deprivation amblyopia and late surgery to allow for more reliable ptosis measurements. Surgery involves a medial canthoplasty for correction of the blepharophimosis, epicanthus inversus, and telecanthus at three to five years of age, followed a year later by ptosis correction, usually requiring brow suspension. Autogeneous *fascia lata* is not reliable before 3.5 to four years of age; in younger children, silastic slings can be used. For individuals with severe ptosis, surgical ptosis repair is recommended before the age of three years; for individuals with moderate ptosis, correction may be deferred until the age of five years. Ovum donation is the only possible therapy for the female infertility resulting from premature ovarian failure. Endocrinologic and gynecologic follow-up are advised in affected females in whom the BPES type is unknown or in whom BPES type I is suspected.

Genetic counseling. BPES is inherited in an autosomal dominant manner. Some individuals diagnosed with BPES have an affected parent. A proband with BPES may have the disorder as the result of a *de novo* gene mutation. The proportion of cases caused by *de novo* mutations is estimated to be more than 50%. Each child of an individual with BPES has a 50% chance of inheriting the mutation. Prenatal testing is available; however, requests for prenatal testing for conditions such as BPES are not common.

Diagnosis

Clinical Diagnosis

The diagnosis of blepharophimosis syndrome (BPES) is based primarily on four clinical findings, which are present at birth [Oley & Baraitser 1995]:

- **Blepharophimosis:** Narrowing of the horizontal aperture of the eyelids. In normal adults, the horizontal palpebral fissure measures 25-30 mm; in individuals with BPES, it generally measures 20-22 mm.
- **Ptosis:** Drooping of the upper eyelid causing a narrowing of the vertical palpebral fissure. In individuals with BPES, ptosis is secondary to dysplasia of the *musculus levator palpebrae superioris*. To compensate for the ptosis, affected individuals:
  - Use the *musculus frontalis*, wrinkling the forehead to draw the eyebrows upward, which results in a characteristic facial appearance
  - Tilt their head backward into a chin-up position
- **Epicanthus inversus:** A skinfold arising from the lower eyelid and running inwards and upwards.
- **Telecanthus:** Lateral displacement of the inner canthi with normal interpupillary distance.

Two types of blepharophimosis syndrome have been described [Zlotogora et al 1983]:

- **BPES type I** includes the four major features and female infertility caused by premature ovarian failure (POF).
- **BPES type II** includes only the four major features.

Testing

Females with premature ovarian failure have endocrinologic findings of hypergonadotrophic hypogonadism:

- Elevated serum concentration of follicle-stimulating hormone (FSH) and luteinizing hormone (LH)
- Decreased serum concentration of estradiol and progesterone

Ultrasonography reveals a small hypoplastic uterus and streak ovaries. Typical anatomic pathologic findings of the ovary are "resistant-ovary syndrome" (presence of primordial follicles, but no follicular development) progressing into a "true premature menopause" (presence of scars in place of primordial follicles) [Fraser et al 1988].

Molecular Genetic Testing

GeneReviews designates a molecular genetic test as clinically available only if the test is listed in the GeneTests Laboratory Directory *by either a US CLIA-licensed laboratory or a non-US clinical laboratory. GeneTests does not verify laboratory-submitted information or warrant any aspect of a laboratory's licensure or performance. Clinicians must communicate directly with the laboratories to verify information.*—ED.

Molecular Genetic Testing—Gene. *FOXL2* is the only gene currently known to be associated with blepharophimosis syndrome.

Molecular genetic testing: Clinical uses

- Confirmatory diagnostic testing

- Prenatal diagnosis

Molecular genetic testing: Clinical methods

- **Sequence analysis of the single coding exon of *FOXL2*.** All intragenic *FOXL2* mutations identified to date are confined to this exon [De Baere et al 2003, Beysen et al 2004].
- **Deletion detection using multiplex-ligation dependent probe amplification (MPLA) and fluorescence in situ hybridization (FISH).** MLPA and FISH with *FOXL2*-containing BAC RP11-548O1 may be used to detect subtle partial/total deletions or microdeletions encompassing *FOXL2* in individuals in whom sequence analysis does not reveal a mutation. Frequencies of *FOXL2* (micro)deletions resulting in BPES are estimated to be about 10% of all molecular defects in individuals with BPES [Beysen et al 2005].
- The detection rate of the combined approach consisting of sequence and FISH analysis is around 70% in familial as well as in simplex cases [De Baere et al 2003, Beysen et al 2005].

Molecular genetic testing: Research

- **Microsatellite and SNP analysis.** Genotyping with more than 60 microsatellites and SNPs may be used to detect rearrangements (microdeletions) located outside the transcription unit of *FOXL2* in individuals who do not have an intragenic mutation or deletion encompassing the gene, estimated to be about 5% of all molecular defects in individuals with BPES [Beysen et al 2005].

Table 1 summarizes molecular genetic testing for this disorder.

Table 1. Molecular Genetic Testing Used in Blepharophimosis, Ptosis, and Epicanthus Inversus

Test Method	Mutations Detected	Mutation Detection Rate	Test Availability
Sequence analysis	Mutations in open reading frame of *FOXL2* [1]	70%	Clinical Testing
Deletion detection (MLPA)	Total gene deletions/microdeletions/submicroscopic deletions encompassing *FOXL2*	10%	
Microsatellite and SNP analysis	Extragenic rearrangements (deletions)	5%	Research only

1. All intragenic mutations identified to date are in the single exon 1 (containing the entire coding region).

Interpretation of test results. For issues to consider in interpretation of sequence analysis results, click here.

Testing Strategy for a Proband

- Depending on the family history and the individual's phenotype, chromosome analysis may be considered if no *FOXL2* mutation or microdeletion of the *FOXL2* region can be identified.
- Cytogenetic testing should particularly be pursued in individuals with BPES who have developmental delay and associated features (see Genetically Related Disorders).

Genetically Related (Allelic) Disorders

Deletion of 3q23. Individuals with BPES caused by cytogenetic rearrangements involving 3q23 (i.e., translocations and interstitial deletions) have been reported [Fukushima et al 1991, Jewett et al 1993, Boccone et al 1994, Lawson et al 1995, Praphanphoj et al 2000, Engelen et al 2002].

Nonsyndromic premature ovarian failure (POF). Considering that POF is part of the phenotypic spectrum of *FOXL2* mutations, *FOXL2* was assumed to be a possible candidate gene for nonsyndromic POF [Crisponi et al 2001, Prueitt & Zinn 2001]. However, the results of the following studies demonstrate that mutations in the *FOXL2* coding region are rarely associated with nonsyndromic POF.

No disease-causing *FOXL2* mutations were identified in 100 unrelated women with POF [De Baere et al 2001, De Baere et al 2002] or in 120 otherwise phenotypically normal women with POF [Bodega et al 2004].

FOXL2 mutations were identified in two of 70 women with POF from New Zealand and Slovenia [Harris et al 2002]. Neither of the two mutations was identified in more than 200 control chromosomes.

- A novel 30-bp deletion that was predicted to remove ten of 14 alanines from the polyAla tract (c.661_690del; g.898_927del; p.A221_A230del) was identified in a Slovenian woman who had spontaneously conceived and delivered two healthy children despite primary amenorrhea and hypergonadotrophic hypogonadism [Gersak et al 2004].
- A novel single nucleotide substitution c.772T↓A (g.1009T↓A; p.Y258N) was identified in a New Zealand woman with POF and her unaffected mother.

Clinical Description

Natural History

Blepharophimosis syndromes type I and II are a complex eyelid malformation characterized by four major features, all present at birth: blepharophimosis, ptosis, epicanthus inversus, and telecanthus.

Associated ophthalmic manifestations include dysplastic eyelids (lack of eyelid folds and thin skin); S-shaped border of the upper eyelid and abnormal downward concavity of the lower eyelid with lateral ectropion; and nasolacrimal drainage problems caused by lateral displacement, duplication, or stenosis of the lacrimal puncta. Nystagmus and microphthalmos can occur. A recent retrospective study in 204 individuals with BPES showed occurrence of manifest strabismus in 20%, a significant refractive error in 34%, and bilateral or unilateral amblyopia in 21% and 20% respectively [Dawson et al 2003]. The incidences of amblyopia, strabismus, and refractive errors (anisometropic hypermetropia and myopia) are higher in individuals with BPES than in the general population [Beckingsale et al 2003, Dawson et al 2003].

Individuals with BPES who have an intragenic disease-causing mutation are expected to have normal intelligence.

Minor features observed in BPES are a broad nasal bridge, low-set ears, and a short philtrum.

In BPES type I, menarche is usually normal, followed by oligomenorrhea and secondary amenorrhea. Secondary sexual characteristics are usually normal.

Genotype-Phenotype Correlations

- Initial studies suggested *FOXL2* haploinsufficiency as the cause of BPES and demonstrated a preliminary genotype-phenotype correlation [Crisponi et al 2001, De Baere et al 2001].

- For some *FOXL2* mutations, intra- and interfamilial variable expressivity of the development of POF has been observed [De Baere et al 2003].
- A revised genotype-phenotype correlation has been proposed:
  - Mutations predicted to result in proteins truncated before the polyalanine tract may lead to POF (BPES type I).
  - Polyalanine expansions may lead to BPES type II.
  - For mutations leading to a truncated or extended protein containing an intact forkhead and polyalanine tract, no correlations can be made.
  - For missense mutations (mainly in the highly conserved forkhead domain), no correlations can be made.
  - For (micro)deletions encompassing *FOXL2*, no reliable genotype-phenotype correlation can be made for psychomotor development. Two individuals with a total gene deletion had BPES type I. Because of the small sample size, this correlation has to be evaluated in additional individuals who have (micro)deletions [Beysen et al 2005].
  - Extragenic rearrangements have no association with psychomotor retardation; correlation with POF risk is as yet unknown [Beysen et al 2005].

Penetrance

- To date, all individuals found to have a *FOXL2* mutation have the BPES phenotype.
- For some *FOXL2* mutations, inter- and intrafamilial variable expressivity of female infertility (premature ovarian failure) is observed. [De Baere et al 2003].

Prevalence

The exact prevalence of BPES is unknown. No differences in prevalence based on sex, race, or ethnicity have been reported.

Differential Diagnosis

For current information on availability of genetic testing for disorders included in this section, see GeneTests Laboratory Directory. —ED.

The differential diagnosis of BPES includes those conditions in which ptosis or blepharophimosis is a major feature (Table 2) [Oley & Baraitser 1995]; however, in clinical practice, blepharophimosis syndrome can be relatively easily distinguished from most of these conditions.

Table 2. Overview of Conditions in which Ptosis and/or Blepharophimosis are Prominent Features

Syndrome	Inheritance [1]	Characteristics	OMIM
Hereditary congenital ptosis 1 (PTOS1)	AD	• Ptosis	178300
Hereditary congenital ptosis 2 (PTOS2)	XL	• Ptosis	300245

Ohdo blepharophimosis syndrome	AD [2]	• Blepharophimosis • Blepharoptosis • Mental retardation • Congenital heart defects • Hypoplastic teeth	249620
Michels syndrome		• Blepharophimosis • Blepharoptosis • Epicanthus inversus • Ophthalmic anterior segment defects (cornea) • Cleft lip/palate • Minor skeletal abnormalities	257920
Ptosis with external ophthalmoplegia	AR	• Ptosis • Ophthalmoplegia • Miosis • Decreased accommodation • Strabismus • Amblyopia	258400
Noonan syndrome	AD	• Ptosis • Short stature • Heart defects • Blood clotting deficiencies	163950
Marden-Walker syndrome	AR	• Ptosis • Blepharophimosis • Growth retardation • Neurologic defects (mental retardation, absent primitive reflexes)	248700
Schwartz-Jampel syndrome		• Intermittent ptosis • Blepharophimosis • Telecanthus • Cataract • Short stature • Cartilage and skeletal anomalies • Muscle hypertrophy	255800
Dubowitz syndrome		• Ptosis • Blepharophimosis • Lateral telecanthus • Short stature • Mental retardation • Immunologic deficiencies	223370

Smith-Lemli-Opitz syndrome		• Ptosis • Epicanthus • Cataract • Growth and mental retardation • Severe genitourinary, cardiac, and gastrointestinal anomalies	270400

Oley & Baraitser 1995, OMIM

1. AD=autosomal dominant; AR=autosomal recessive, XL=X-linked
2. Presumed mode of inheritance

Management

Evaluations at Initial Diagnosis to Establish the Extent of Disease

- Individuals should be thoroughly examined by a (pediatric) opthalmologist for visual acuity measurement, refraction, measurement of ocular movements and strabismus, and measurement of palpebral apertures and eyelid elevation.
- Individuals with evidence of amblyopia or strabismus should be referred to a pediatric ophthalmologist for appropriate management [Beckingsale et al 2003].
- Individuals with BPES should be referred to a clinical geneticist for an appropriate genetic workup and counseling. In girls affected with BPES, the family history can already give an indication of the type of BPES (association with subfertility or infertility in affected females). In uninformative families or simplex cases (i.e., in which only one family member is affected), molecular genetic testing may be helpful for assessing for premature ovarian failure risk in some cases.

Treatment of Manifestations

Management requires the input of several specialists including a geneticist, pediatric ophthalmologist, orthoptist, oculoplastic surgeon, pediatric endocrinologist, reproductive endocrinologist, and gynecologist.

Timing of eyelid surgery is controversial; it involves weighing the balance of early surgery to prevent deprivation amblyopia and late surgery to allow for more reliable ptosis measurements, the latter of which provides a better surgical outcome. Surgery is hampered by the dysplastic structure of the eyelids [Beckingsale et al 2003]. The surgical management traditionally involves a medial canthoplasty for correction of the blepharophimosis, epicanthus inversus, and telecanthus at three to five years of age, followed about a year later by ptosis correction, which usually requires a brow suspension procedure. Autogeneous *fascia lata* gives excellent results, but is not reliable before 3.5 to four years of age because of a lack of available autogeneous *fascia lata*. In children below this age, silastic slings give good results and are easily adjusted if necessary.

- For individuals with severe ptosis, surgical ptosis repair is recommended before the age of three years, followed by medial canthoplasty if necessary.
- For individuals with moderate ptosis, correction of ptosis may be deferred until the age of five years when surgery is often recommended for cosmetic reasons before starting school.

Management of POF needs to address the two major medical issues: hormone replacement therapy (HRT) and infertility.

- **HRT.** Estrogen and progesterone replacement therapy is usually indicated. No comparative data are available to guide estrogen use in young women as most studies on HRT involve post-menopausal women [Goswami & Conway 2005].
- **Infertility.** No effective treatment for infertility exists. Adoption and oocyte donation are among the available options but require guidance and counseling [Hovav et al 1995, Goswami & Conway 2005].

Surveillance

- The frequency of ophthalmic follow-up is variable depending on the individual's age, procedures performed, and results of visual testing.
- Endocrinologic and gynecologic follow-up are advised in affected females in whom the BPES type is unknown or in whom BPES type I is suspected based on a positive family history or suggestive *FOXL2* mutation. Monitoring of the ovarian status can be done by ultrasonography of ovaries and uterus, hormonal measurements (FSH, LH, estrogen, progesterone), and following the natural history of menses (age of menarche and ages of onset of oligomenorrhea and secondary amenorrhea).

Therapies Under Investigation

Ovarian tissue and oocyte cryopreservation hold promise for fertility preservation in the women most likely to undergo ovarian failure (reviewed in Goswami & Conway 2005). Adolescent girls with BPES who have a risk of developing POF could be candidates for ovarian cryopreservation. Cryopreserved ovarian tissue could be used in two ways: autograft and in vitro folliculo-oocyte maturation. Cryopreserved human ovarian tissue has been found to be functional after retransplantation. The first live birth after orthoptic transplantation of cryopreserved ovarian tissue was reported recently [Donnez et al 2004].

Note: (1) Cryopreservation has not yet been reported in BPES. (2) Children who are at risk for POF are most likely to benefit from cryopreservation as their ovaries contain more primordial follicles than those of adult women; it is expected that by the time these children are mature and need their ovarian tissue, the modalities for its optimal use would become available. (3) At the time that they might wish to consider an IVF procedure, adult women with BPES usually do not have sufficient appropriate primordial follicles for embryo cryopreservation.

Search ClinicalTrials.gov for access to information on clinical studies for a wide range of diseases and conditions.

Genetic Counseling

Genetic counseling is the process of providing individuals and families with information on the nature, inheritance, and implications of genetic disorders to help them make informed medical and personal decisions. The following section deals with genetic risk assessment and the use of family history and genetic testing to clarify genetic status for family members. This section is not meant to address all personal, cultural, or ethical issues that individuals may face or to substitute for consultation with a genetics professional. To find a genetics or prenatal diagnosis clinic, see the GeneTests Clinic Directory.

Mode of Inheritance

Blepharophimosis, ptosis, and epicanthus inversus syndrome is inherited in an autosomal dominant manner.

Risk to Family Members

Parents of a proband

- Some individuals diagnosed with BPES have an affected parent.
- A proband with BPES may have the disorder as the result of a *de novo* gene mutation. The proportion of cases caused by *de novo* mutations is estimated at more than 50% [unpublished data].
- Recommendations for the evaluation of parents of a proband with an apparent *de novo* mutation include molecular genetic testing of the *FOXL2* gene if a mutation has been identified in the proband and clinical examination for subtle features of BPES.

Note: Variable expressivity of BPES features has only been reported in mosaic cases [unpublished data].

Sibs of a proband

- The risk to the sibs of the proband depends upon the genetic status of the proband's parents.
- If a parent of the proband is affected, the risk to the sibs is 50%.
- When the parents are clinically unaffected and do not have a *FOXL2* mutation, the risk to the sibs of a proband appears to be low.
- If a disease-causing *FOXL2* mutation cannot be detected in the DNA of either parent, two possible explanations are germline mosaicism in a parent or a *de novo* mutation in the proband. The risk to the sibs of the proband depends on the probability of germline mosaicism in a parent of the proband and the spontaneous mutation rate of *FOXL2*.
- Germline mosaicism has been observed in BPES and demonstrated at the molecular level [Beysen et al 2005]; its incidence is unknown.

Offspring of a proband. Each child of an individual with BPES has a 50% chance of inheriting the mutation.

Other family members of a proband. The risk to other family members depends upon the genetic status of the proband's parents. If a parent is found to be affected, his or her family members are at risk.

Related Genetic Counseling Issues

Considerations in families with an apparent *de novo* mutation. When neither parent of a proband with an autosomal dominant condition has the disease-causing mutation or clinical evidence of the disorder, it is likely that the proband has a *de novo* mutation. However, possible non-medical explanations including alternate paternity or undisclosed adoption could also be explored.

Family planning. The optimal time for determination of genetic risk and dicussion of the availability of prenatal diagnosis is before pregnancy.

DNA banking. DNA banking is the storage of DNA (typically extracted from white blood cells) for possible future use. Because it is likely that testing methodology and our understanding of genes, mutations, and diseases will improve in the future, consideration should be given to banking DNA of affected individuals. DNA banking is particularly relevant

in situations in which the sensitivity of currently available testing is less than 100%. See DNA Banking for a list of laboratories offering this service.

Prenatal Testing

Prenatal diagnosis for pregnancies at increased risk is possible by analysis of DNA extracted from fetal cells obtained by amniocentesis usually performed at about 15-18 weeks' gestation or chorionic villus sampling (CVS) at about 10-12 weeks' gestation. The disease-causing allele of an affected family member must be identified before prenatal testing can be performed.

Note: Gestational age is expressed as menstrual weeks calculated either from the first day of the last normal menstrual period or by ultrasound measurements.

Requests for prenatal testing for conditions such as BPES are not common. Differences in perspective may exist among medical professionals and within families regarding the use of prenatal testing, particularly if the testing is being considered for the purpose of pregnancy termination rather than early diagnosis. Although most centers would consider decisions about prenatal testing to be the choice of the parents, careful discussion of these issues is appropriate.

Preimplantation genetic diagnosis (PGD) may be available for families in which the disease-causing mutation has been identified in an affected family member in a research or clinical laboratory. For laboratories offering PGD, see Testing.

Molecular Genetics

Information in the Molecular Genetics tables is current as of initial posting or most recent update. —ED.

Table A. Molecular Genetics of Blepharophimosis, Ptosis, and Epicanthus Inversus

Gene Symbol	Chromosomal Locus	Protein Name
FOXL2	3q23	Forkhead box protein L2

Data are compiled from the following standard references: Gene symbol from HUGO; chromosomal locus, locus name, critical region, complementation group from OMIM; protein name from Swiss-Prot.

Table B. OMIM Entries for Blepharophimosis, Ptosis, and Epicanthus Inversus

110100	BLEPHAROPHIMOSIS, EPICANTHUS INVERSUS, AND PTOSIS; BPES
605597	FORKHEAD TRANSCRIPTION FACTOR FOXL2; FOXL2

Table C. Genomic Databases for Blepharophimosis, Ptosis, and Epicanthus Inversus

Gene Symbol	Locus Specific	Entrez Gene	HGMD
FOXL2	FOXL2	668 (MIM No. 605597)	FOXL2

For a description of the genomic databases listed, click here.

Normal allelic variants: The *FOXL2* gene is a small single-exon gene of 2.7 kb. The entire open reading frame is highly conserved in several vertebrate species [Cocquet et al 2002, Cocquet et al 2003, Udar et al 2003].

Pathologic allelic variants: Over 125 *FOXL2* mutations have been described in individuals with BPES types I and II, demonstrating that both phenotypic features (eyelid defect and POF) are caused by the pleiotropic effect of a single gene, rather than by a contiguous gene syndrome. A locus-specific Human FOXL2 Mutation Database contains general information about the *FOXL2* gene, as well as details about 135 intragenic mutations and variants of *FOXL2* obtained

from published papers, abstracts of meetings, and unpublished data. Not included in the current version of the database are variants residing outside the coding region of *FOXL2* and molecular cytogenetic rearrangements of the *FOXL2* locus [Beysen et al 2004].

Haploinsufficiency of *FOXL2* appears to be the cause of BPES as 80% of mutations are either intragenic mutations (polyalanine expansions or frameshift, missense, or nonsense mutations) or partial/total deletions of *FOXL2* or microdeletions and submicroscopic deletions encompassing *FOXL2* and neighboring genes [De Baere et al 2001, De Baere et al 2003, Beysen et al 2005]. Two mutational "hot spots" have been demonstrated: 30% of *FOXL2* mutations lead to polyalanine expansions, and 13% are a 17-bp duplication c.843_859dup (g. 1080-1096dup; p.P287fs) [De Baere et al 2003].

A recent study showed that genomic rearrangements encompassing *FOXL2* account for 10% of all molecular defects found in BPES families.

In a four-generation Chinese family with BPES type II showing linkage to the *FOXL2* locus, an insertion mutation in the 3' UTR of *FOXL2* segregated with the phenotype. However, the functional significance of this 3' UTR insertion on *FOXL2* transcript stability and translation still needs to be proven [Qian et al 2004].

The occurrence of three translocation breakpoints located upstream of *FOXL2* illustrated that a position effect may also be implicated in the causation of BPES. Beysen et al (2005) reported on five extragenic deletions in individuals with typical features of BPES, providing further evidence of potential long-range cis-regulatory elements regulating *FOXL2* expression. The rearrangements outside the transcription unit are estimated to account for 5% of all molecular defects found in BPES [Beysen et al 2005].

Note: Following the most recent HGVS guidelines, mutations are named in three different ways: common, systematic, and trivial. What have been classified as "common" names are the mutations numbered using the reference sequence AF301906 (initiated by Crisponi et al 2001). The common names are preceded by a "g." following the HGVS recommendations. The "systematic" names have been numbered using the cDNA reference numbering with +1 as A of the initation codon ATG. The "trivial" names reflect the mutations on the protein level and the notation has been adapted following the most recent guidelines.

Normal gene product: The FOXL2 protein of 376 amino acids belongs to the large family of winged-helix/forkhead transcription factors. Forkhead proteins are present in all eukaryotes and have important functions in the establishment of the body axis and the development of tissues from all three layers in animals. Apart from the following two domains no similarities to other known proteins or domains have been identified [Crisponi et al 2001].

- FOXL2 also contains a characteristic DNA-binding domain of 110 amino acids that was originally identified in *Drosophila melanogaster fork head* mutant; the domain was nearly perfectly conserved between *fork head* and the mammalian HNF-3 transcription factors [Weigel et al 1990].
- FOXL2 contains a polyalanine tract of 14 residues, the role of which has not yet been elucidated. Expansions from 14 to 24 alanine residues in this region represent about 30% of all intragenic *FOXL2* mutations and lead mainly to BPES type II [De Baere et al 2003].

  (Follow links to Box 1 detailed information.)

  - Forkhead transcription factor
  - Evolution

- Expression
- Subcellular localization
- Protein interactions

Abnormal gene product: Promoter reporter studies revealed that *FOXL2* is a transcriptional repressor of the *StAR* gene, a marker of granulosa cell differentiation. DNA-binding studies revealed that *FOXL2* directly interacts with the first 95 bp upstream of the start site of the *StAR* promoter. *FOXL2* mutants that lack the entire alanine/proline-rich carboyl-terminus and that have been found in individuals with BPES with POF show loss of repressor activity. In addition, they exhibit a dominant-negative effect by blocking the repressor activity of wild-type *FOXL2*. It has been concluded that *FOXL2* mutations resulting in BPES type I may be associated with accelerated differentiation of granulosa cells and secondary depletion of the primordial follicle pool [Pisarska et al 2004].

Caburet et al (2004) studied the subcellular localization of the recurrent polyalanine expansion p.A224_A234dup by transfecting COS-7 cells with DNA constructs driving the expression of the wild-type and mutant *FOXL2* proteins fused to the green fluorescent protein (GFP). The polyAla expansion was found to induce the formation of intranuclear aggregates and a mislocalization of the protein as a result of extensive cytoplasmic aggregation. Cotransfection experiments suggested that the wild-type and mutant proteins can co-aggregate. It was proposed that the pathogenic mechanism of the *FOXL2* polyAla expansions may be its mislocalization together with its inclusion into nuclear aggregates. Together with the study by Albrecht et al (2004) this study was the first to propose a mechanism for the molecular pathogenesis of polyAla repeat expansions in transcription factors [Caburet et al 2004].

In vivo studies: *(Follow links to Box 2 for detailed information.)*

- Polled intersex syndrome (PIS) in goat
- Mouse models
- Structure, evolution, and expression

Resources

GeneReviews provides information about selected national organizations and resources for the benefit of the reader. GeneReviews is not responsible for information provided by other organizations. Information that appears in the Resources section of a GeneReview is current as of initial posting or most recent update of the GeneReview. Search GeneTests*for this disorder and select* Resources *for the most up-to-date Resources information.*—ED.

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Email: info@aboutfaceinternational.org
www.aboutfaceinternational.org

Children's Craniofacial Association
13140 Coit Road, Suite 307
Dallas, TX 75240
Phone: 800-535-3643; 214-570-8811

Email: contactCCA@ccakids.com
www.ccakids.com

FACES: The National Craniofacial Association
PO Box 11082
Chattanooga, TN 37401
Phone: 800-332-2373; 423-266-1632
Email: faces@faces-cranio.org
www.faces-cranio.org

Forward Face, Inc
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Society for the Rehabilitation of the Facially Disfigured
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References

Medical Genetic Searches: A specialized PubMed search designed for clinicians that is located on the PubMed Clinical Queries page. PubMed

Published Statements and Policies Regarding Genetic Testing

No specific guidelines regarding genetic testing for this disorder have been developed.

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Chapter Notes

Revision History

- 15 February 2006 (cd) Revision: prenatal diagnosis available
- 12 July 2005 (me) Comprehensive update posted to live Web site
- 8 July 2004 (me) Review posted to live Web site
- 1 March 2004 (edb) Original submission

Normal Gene Product

Forkhead transcription factor

More than 20 human forkhead genes are known and several have been implicated in tumorigenesis (see review in Carlsson & Mahlapuu 2002). So far, mutations in eight different forkhead genes have been associated with human developmental disorders. Their phenotypes are pleiotropic and include ocular, craniofacial, circulatory, skeletal, immune and gonadal defects. Four of the disorders include eye abnormalities (see review in Lehmann et al 2003). Another member of the forkhead family, FOXO3a, has been shown to act as a suppressor of follicular activation as knockout mice develop a premature ovarian failure phenotype [Castrillon et al 2003]. Brunet et al (1999) previously demonstrated that the activation of many transcription factors involved in apoptosis can regulate the expression of *FOXO3a*, which may link *FOXO3a* to apoptosis of oocytes.

Evolution

A comparative analysis between seven mammalian and three non-mammalian vertebrate species showed that the entire open reading frame is under purifying selection leading to strong protein conservation in several species [Cocquet et al 2002, Cocquet et al 2003]. More specifically it was shown that the number of alanine residues is strictly conserved among the mammals studied, suggesting the existence of strong functional or structural constraints The sequence and properties of FOXL2 forkhead domain are highly conserved, which is a general characteristic of forkhead transcription factors [Carlsson & Mahlapuu 2002].

Expression

The expression of the FOXL2 transcript and protein has been studied in mammals: in human, mouse, and goat, a spatially and temporarily restricted expression pattern has been demonstrated in developing eyelids and in fetal and adult ovaries [Crisponi et al 2001, Cocquet et al 2002, Bodega et al 2004].

Ovarian expression is confined to granulosa cells, (and not in the oocytes), supporting a maintenance function.

The pattern of expression of the FOXL2 protein in human developing eyelids reveals that FOXL2 is expressed in the bulging and surrounding primordial mesenchyma, suggesting a role in the development of extraocular muscles consistent with an absence or hypotrophy of the *musculus levator palpebrae superioris* described in individuals with BPES [Dollfus et al 2003]. Its protein localization has been shown to be nuclear, which is in line with its putative function as a transcription factor [Cocquet et al 2002].

Mouse *Foxl2* transcript (previously named *P-Frk* for pituitary forkhead factor) was initially found to be expressed in Rathke's pouch of the developing pituitary gland [Treier et al 1998].

Loffler et al (2003) showed *FoxL2* expression at an early stage in the developing female gonad and a sex-dimorphic expression in gonads of non-mammalian vertebrates (chicken, turtle).

In a similar study, chicken *cFoxL2* expression was studied in developing and adult gonads to examine the role of *FOXL2* in ovarian differentiation and function in birds [Govoroun et al 2004]. The spatial and temporal dynamics of *cFoxL2* and aromatase expression were analyzed in parallel to investigate the possible role of *cFoxL2* in the regulation of aromatase. The expression patterns of *cFoxL2* and aromatase transcripts were highly correlated during the sex-differentiation period. Aromatase and *cFoxL2* proteins were colocalized in the medullar part of female gonads on embryonic day 14. After fourteen days, *cFoxL2* protein was mainly detected in granulosa cells of developing follicles. In adult ovary follicular envelopes, *cFoxL2* transcript and protein were detected, apart from granulosa cells, at lower levels in theca cells where aromatase was present. A high level of *cFoxL2* transcription was also observed in maturing and ovulated oocytes. These results confirmed that *FoxL2* is an early regulator of ovarian development in birds and may be involved in aromatase transcription regulation [Govoroun et al 2004].

The conservation of *FoxL2* sequence and pattern of expression throughout vertebrate evolution leads to the conclusion that it is, to date, the earliest known sex-dimorphic marker of ovarian determination/differentiation in vertebrates and may be a key factor in the early development and maintenance of the vertebrate female gonad. *FoxL2* is the first human autosomal gene shown to be implicated in ovarian maintenance. In addition, its pituitary expression has suggested an involvement in pituitary organogenesis.

A *FoxL2* cDNA was cloned from the Nile tilapia ovary. Alignment of known *FoxL2* sequences from vertebrates confirmed the conservation of the *FoxL2* open reading frame and protein sequences, especially the forkhead domain and C-terminal region, while some homopolymeric runs of amino acids are found only in mammals, not in non-mammalian vertebrates. *FoxL2* was shown to be expressed in the tilapia brain (B), pituitary (P), gill, and gonads (G), with the highest level of expression in the ovary, reflecting the involvement of *FoxL2* in B-P-G axis and revealing a sexual dimorphic expression pattern in the gonads. *FoxL2* mRNA was mainly detected in the granulosa cells surrounding the oocytes. The ovarian expression of *FoxL2* in tilapia begins early during the differentiation of the gonads and persists until adulthood, implying the involvement of *FoxL2* in fish gonad differentiation and the maintenance of ovarian function [Wang et al 2004].

Sd-FoxL2 was cloned in the sponge *Suberites domuncula*, consisting of a 1266-bp long cDNA and of 275 aa. The phylogenetic analysis of its forkhead domain classifies it clearly

within the group of the FoxL2 proteins, very closely related to the human FoxL2. Its alignment with the human FoxL2 protein shows a highly conserved forkhead domain and a quite conserved carboxy-half part with a high content of proline and alanine residues. Immunohistochemistry showed that Sd-FoxL2 is detected in the nucleus of all cells in the sponge tissue and in the primmorphs. This ubiquitous expression pattern was surprising compared to that seen in vertebrates. Its expression in gemmules indicated a vital function of Sd-FoxL2 for sponge cells [Adell & Muller 2004].

Subcellular localization

In situ hybridization [Crisponi et al 2001] and immunohistochemistry [Cocquet et al 2002] have demonstrated that FOXL2 is a nuclear protein expressed in eyelids and in fetal and adult ovarian follicular cells that does not undergo any important post-translational maturation.

Protein interactions

At the level of protein interactions, FoxL2 has been shown to be capable of interacting at GRAS, or **G**nRH **r**eceptor **a**ctivating **s**equence, which is a regulatory element of the murine GnRH receptor gene promoter mediating activin responsiveness. FoxL2 activation at GRAS is lost with mutation of either the 5' Smad binding site or a putative forkhead binding site located at the 3' end of the element. It has been suggested that GRAS is a composite regulatory element whose functional activity is dependent on the organization of a multi-protein complex consisting of Smads, AP-1, and a member of the forkhead family of DNA-binding proteins [Ellsworth et al 2003].

Abnormal Gene Product

Polled intersex syndrome (PIS) in goat

In mammals, the Y-located *SRY* gene is known to induce testis formation from the indifferent gonad. A related gene, *SOX9*, also plays a critical role in testis differentiation in mammals, birds, and reptiles. It is now assumed that *SRY* acts upstream of *SOX9* in the sex determination cascade, but the regulatory link which should exist between these two genes remains unknown. Studies on XX sex reversal in polled intersex syndrome (PIS) goats have led to the discovery of a female-specific locus crucial for ovarian differentiation. In the PIS goats, dominant polledness is associated with recessive XX intersexuality. The PIS locus was shown to be located at goat chromosome 1q43, a region syntenic to human chromosome band 3q23 [Vaiman et et al 1999]. Despite important phenotypic differences, the PIS goat has been considered to be an animal model for BPES. Pailhoux et al (2001) have shown that PIS is caused by the deletion of a critical 11.7-kb DNA element devoid of coding sequences and containing several repetitive elements. However, it was demonstrated that the deletion affects the transcription of at least two genes: the non-coding *PISRT1* gene (PIS-regulated transcript number 1), and *FOXL2*, located at 20 kb and 200 kb with respect to the deletion, respectively. This suggested a common long-range transcriptional regulation of *PISRT1* and *FOXL2* expression in the goat by the PIS locus and is suggestive of a putative disease-causing mechanism for BPES in humans.

Mouse models

A recent study presenting homozygous *Foxl2lacZ* mutant mice has shed light on the function of *FOXL2* during folliculogenesis in vivo. In *Foxl2lacZ*, granulosa cells from homozygous mutant ovaries do not complete the squamous-to-cuboidal transition, leading to the absence of secondary follicles and oocyte atresia. Activin-bA and anti-Mullerian inhibiting hormone expression is absent or strongly diminished in *Foxl2lacZ* homozygous mutant ovaries. Two weeks after birth, most, if not all, oocytes expressed Gdf9 in *Foxl2lacZ* homozygous mutant

ovaries, indicating that nearly all primordial follicles have already initiated folliculogenesis at this stage. This activation, in the absence of functional granulosa cells, leads to oocyte atresia and progressive follicular depletion. In addition to providing a molecular mechanism for premature ovarian failure in BPES, this study showed that granulosa cell function is not only crucial for oocyte growth but also for ovary maintenance in vivo [Schmidt et al 2004]. A second report confirmed that mice lacking *Foxl2* recapitulate features of human BPES and that granulosa cell development fails in *Foxl2*-null animals from the time of primordial follicle formation [Uda et al 2004].

Structure, evolution, and expression

Crisponi et al (2004) reported that three translocation breakpoints, located at more than 171 kb 5' of the transcription start of *FOXL2* causing BPES, fall within intron 6 of *MRPS22*, a large gene consisting of 20 exons. Sequence comparisons revealed conserved segments in introns 6, 11, and 12 of human and mouse. The intron 11 sequence is also deleted in the PIS goat. The authors stated that the conserved sequences are candidates to be distant enhancers or otherwise to affect higher-order chromatin structure to impose long-range *cis*-regulation of FOXL2 expression.

A comparative human/mouse analysis of the orthologous region around the PIS locus by Nikic and Vaiman (2004) permitted the targeting of genes in the 1-mb environment, and identification of previously unknown mouse orthologues for *Pisrt1*, *Bpesc1*, and *Chr3syt*, and a human orthologue for *PISRT1*. Tissue-specific gene expression in mice and goats was assessed for ten and eight genes, respectively, located in a 1-mb DNA region surrounding the PIS locus. It was shown that gene expression is essentially regulated in a similar manner in goat and mouse tissues in the PIS vicinity.

Beysen et al (2005) identified novel microdeletions outside *FOXL2* in simplex and familial BPES cases. Specifically, four rearrangements with an overlap of 126 kb are located 230 kb upstream of *FOXL2*, telomeric to the reported translocation breakpoints. Interestingly, the human orthologous region of a 12-kb sequence deleted in the polled intersex goat is contained in this SRO, providing evidence of human-goat conservation of *FOXL2* expression and of the mutational mechanism.