## Blepharophimosis, Ptosis, and Epicanthus Inversus

## *FOXL2* 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].

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