FOXL2 Impairment in Human Disease

Hannah Verdin Elfride De Baere

Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium

Key Words

FOXL2 mutation • BPES • POF • Adult GCT • Anti-testis action

Abstract

FOXL2 encodes a forkhead transcription factor that plays important roles in the ovary during development and in postnatal, adult life. Here, we focus on the clinical consequences of *FOXL2* impairment in human disease. In line with other forkhead transcription factors, its constitutional genetic defects and a somatic mutation lead to developmental disease and cancer, respectively. More than 100 unique constitutional mutations and regulatory defects have been found in blepharophimosis syndrome (BPES), a complex eyelid malformation associated (type I) or not (type II) with premature ovarian failure (POF). In agreement with the BPES phenotype, *FOXL2* is expressed in the developing eyelids and in fetal and adult ovaries. Two knock-out mice and at least one natural animal model, the Polled Intersex Syndrome goat, are known. They recapitulate the BPES phenotype and have provided many insights into the ovarian pathology. Only a few constitutional mutations have been described in nonsyndromic POF. Moreover, a recurrent somatic mutation p.C134W was found to be specific for adult ovarian granulosa cell tumors. Functional studies investigating the consequences of *FOXL2* mutations or regulatory defects have shed light on the molecular pathogenesis of the aforementioned conditions, and contributed considerably to genotype-phenotype correlations. Recently, a conditional knock-out of *Foxl2* in the mouse induced somatic transdifferentiation of ovary into testis in

adult mice, suggesting that *Foxl2* has an anti-testis function in the adult ovary. This changed our view on the ovary and testis as terminally differentiated organs in adult mammals. Finally, this might have potential implications for the understanding and treatment of frequent conditions such as POF and polycystic ovary syndrome.

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Introduction

FOXL2 is a single-exon gene of 2.7 kb encoding a highly conserved protein of 376 amino acids containing a 110-amino-acid DNA-binding forkhead domain, classifying *FOXL2* in the family of forkhead transcription factors involved in a wide variety of biological processes during development and postnatal life. Besides the forkhead domain, *FOXL2* also contains a polyalanine tract of 14 residues that is conserved in mammals; however, the exact function is unknown (fig. 1). As several forkhead transcription factors have a strict spatiotemporal expression pattern in early development, a mutation dysregulating this pattern could lead to developmental disease. Currently, 11 human forkhead genes have been shown to be mutated in human hereditary developmental disorders, four of which have an ocular phenotype [1]. One of them is *FOXL2*, leading to blepharophimosis-ptosis-epicanthus inversus syndrome with or without ovarian dysfunction when mutated (BPES, OMIM 110100) [2]. The expression pattern of *FOXL2* is compatible with the BPES

Fig. 1. Schematic outline of the *FOXL2* gene and protein. *FOXL2* encodes a protein of 376 amino acids. The characteristic forkhead domain and the polyalanine tract are indicated by an arrowhead. Adapted from Beysen et al. [19].

Fig. 2. Complex eyelid malformation in BPES. The four major characteristics of the eyelids are: (1) blepharophimosis or a reduction of the horizontal dimension of the palpebral fissures, (2) ptosis or drooping of the upper eyelid, (3) epicanthus inversus or a skin fold rising from the lower eyelid and running inwards and upwards, and (4) telecanthus or a lateral dislocation of the canthi with normal inter-pupillary distance.

phenotype, as expression studies in human, mouse and goat demonstrated the presence of the nuclear protein in the mesenchyme of developing eyelids and in fetal and adult supporting granulosa cells but not in the oocytes. As *FOXL2* expression in the ovary is observed before folliculogenesis, it is the earliest known marker of ovarian differentiation in mammals. Moreover, *FOXL2* is strongly expressed in adult follicular cells, suggesting not only a role in ovarian somatic cell differentiation but also in adult female fertile life in follicular development and maintenance. *FOXL2* expression has also been demonstrated in the developing pituitary, and in gonadotrope as well as thyrotrope cell types of the adult pituitary, suggesting an involvement in pituitary organogenesis [3–6].

***FOXL2* Impairment in BPES**

Clinical Features of BPES

Complex Eyelid Malformation

BPES is a rare developmental disorder of the eyelids and ovary essentially presenting with an autosomal dominant inheritance. Typically, four major characteristics of

the eyelids are present at birth (fig. 2): (1) a reduction of the horizontal dimension of the palpebral fissures (blepharophimosis), (2) drooping of the upper eyelid leading to a narrowing of the vertical palpebral fissure (ptosis), (3) a small skin fold rising from the lower eyelid and running inwards and upwards (epicanthus inversus), and (4) a lateral displacement of the canthi with normal interpupillary distance (telecanthus). Besides this clinical tetrad, other ophthalmic features associate with BPES including nasolacrimal drainage problems caused by lateral displacement, duplication, or stenosis of the lacrimal puncta. Especially the lateral displacement of the inferior punctum is a relatively unknown and important anatomical hallmark for the clinical diagnosis of BPES [7]. Minor features observed in BPES are a broad nasal bridge, low-set ears and a short philtrum [8]. The eyelid malformation can be corrected with oculoplastic surgery for both aesthetic and functional reasons. Traditionally, a medial canthoplasty to correct the epicanthic folds is performed at the age of 3–5 years depending on the severity of the ptosis, followed by ptosis correction. Decock et al. [7] described two surgical procedures to correct ptosis, i.e. super-maximal resection and frontalis suspension. Indeed,

super-maximal resection of the levator muscle is the preferred method as it leads to a good cosmetic outcome as well as to an improved muscle function.

Ovarian Phenotype

Two types BPES can be clinically distinguished: both types have the clinical tetrad in common while in type I, premature ovarian failure (POF) manifests with the ocular phenotype [9]. POF is defined as amenorrhea for at least 4 months before the age of 40 years, combined with a decreased serum concentration of estradiol and an increased serum concentration of follicle-stimulating hormone (FSH >40 IU/l) [10]. Only little is known about the molecular mechanisms of FOXL2 dysregulation in the ovary of a BPES patient. In a first report by Fraser et al. [11], small hypoplastic uteri and streak ovaries were observed in two sisters with BPES type I (age difference of 8 years) using ultrasonography. On ovarian biopsy, primordial follicles progressing into scars were described. No genetic study was performed however. A second unique study by Meduri et al. [12] included an extensive clinical, genetic, hormonal and ovarian histological investigation in two BPES type I patients. Histological and immunohistological studies were performed on ovarian biopsies from these 2 patients, in which FOXL2 elongating frameshift mutations were found. The ovarian histological phenotype of the first patient was similar to that observed in the knock-out mice, while that of the second patient was apparently normal (fig. 3). In both patients, FOXL2 protein expression was observed in the granulosa cells. In patient 1, a predominant intracytoplasmatic expression was detected, while on the contrary a predominant nuclear expression was seen in patient 2. Taken together, different ovarian phenotypes, follicular defects and distribution of FOXL2 protein were observed in these two patients with molecularly proven defects [12].

In female type I BPES patients, both emotional and physical management of POF is imperative. A first issue in the management of POF is the necessity of hormone replacement therapy to reduce postmenopausal symptoms and prevent long-term health effects of estrogen deficiency, such as an increased risk of osteoporosis [13]. In general, POF reduces the chance of conceiving naturally to 5–10%. Of these pregnancies another 20% result in pregnancy loss [14]. Currently, three fertility-preserving strategies are available: embryo cryopreservation, oocyte cryopreservation and ovarian tissue cryopreservation. The only established method is embryo cryopreservation. This option, however, is restricted to patients who have a partner or are willing to use donor

Fig. 3. Histology of ovarian biopsy of BPES type 1 patient. **a** Numerous, crowded, small follicles are observed in the ovarian cortex. HE. **b** Double oocytes in two small follicles, a third follicle (lower left) is normal. HE. **c** Empty spaces in a small follicle suggesting deposits of cholesterol crystals which have dissolved during fixation leaving cholesterol clefts. Adapted from Meduri et al. [12].

sperm. This also requires a cycle of ovarian stimulation. For those patients without a partner or not willing to use donor sperm, oocyte cryopreservation can be performed. This strategy also requires a cycle of ovarian stimulation. The success rate of oocyte cryopreservation is however still very low with pregnancy rates ranging from 1 to 5% [15, 16]. The aforementioned strategies are not suitable in prepubertal patients however, because of the necessity of ovarian stimulation. Ovarian tissue cryopreservation is in theory possible before puberty. The aim of this technique is to freeze ovarian cortex tissue, and then to reimplant the tissue orthotopically (into the pelvic cavity) or heterotopically (e.g. the forearm or abdominal wall). Orthotopic reimplantation allows the possibility of pregnancy without further reproductive medical assistance. On the contrary, heterotopic reimplantation needs to be followed by in vitro fertilization (IVF). However, because the revascularization after the reimplantation needs time to develop, ischemia damages the follicles resulting in massive follicle loss. Therefore, further research is needed to enhance the freezing and revascularization processes. To date, only 15 live births have been reported, none of which were derived from cryopreserved prepubertal tissue [15–17]. Of note, most BPES type I patients are diagnosed in infancy. Two other options that need to be considered are adoption and oocyte donation. The latter option gives the patient the chance of experiencing a pregnancy herself. Traditionally, fresh oocyte donations were performed, but these are hampered by inefficiency, difficulties of synchronization between recipient and donor, very long waiting periods and lack of quarantine measures. The advances in oocyte cryopreservation techniques with fertilization, implantation and pregnancy rates comparable to fresh oocyte donation, diminishes the disadvantages of fresh oocyte

donation and may become the new standard in the future [18]. The fact that young patients are most often not ready to decide on their reproductive future and that the age of onset of POF cannot be predicted, makes counseling on fertility preservation very challenging. In general, BPES type I patients require a thorough follow-up of ovarian function by both an endocrinologist and gynecologist.

Molecular Genetics of BPES

In 2001, *FOXL2* was identified as the causal gene for BPES [2]. Using a combined mutation detection approach consisting of (1) sequencing of the *FOXL2* open reading frame, (2) copy number screening of the *FOXL2* gene, and (3) copy number screening of the regulatory domain of *FOXL2*, the underlying molecular defect can be identified in 88% of typical BPES patients. Of all genetic defects identified, intragenic mutations represent the largest group (71%). Deletions encompassing *FOXL2* and located outside its transcription unit represent 12 and 5% of molecular defects, respectively [19].

Intragenic Mutations

Intragenic mutations occur along the total coding region of *FOXL2* and all types of mutations have been identified, mostly in BPES. More than 100 unique *FOXL2* mutations have been described (<http://medgen.ugent.be/FOXL2>) [19]. The largest group (44%) contains frameshift mutations. Following are the in-frame changes (33%), the nonsense mutations (12%) and finally the missense mutations (11%). Notably, 93% of the in-frame mutations lead to polyalanine expansions, representing the most important mutational hotspot in *FOXL2* [20].

Several genotype-phenotype correlations emerged after the identification of the first mutations in *FOXL2*. Before any functional studies were done, it was proposed that mutations predicted to result in proteins with truncation before the poly-Ala tract might be associated with BPES type I, whereas polyalanine expansions might rather lead to BPES type II. For missense mutations and mutations leading to a truncated or extended protein containing an intact forkhead domain and polyalanine tract, no correlation could be made [20, 21].

From the first mutation studies, it was hypothesized that these mutations were loss-of-function alleles leading to haploinsufficiency of *FOXL2* [2, 21]. This was supported by the observation that *FOXL2* deletions and intragenic mutations lead to the same phenotype [19]. However, the functional consequences were not clear for missense mutations.

Molecular Consequences of Intragenic Mutations

Most insights into the molecular effects of *FOXL2* mutations contributing to genotype-phenotype correlations resulted from in vitro studies. The most frequent polyalanine expansion p.Ala224_Ala234dup was found to lead to intranuclear aggregation and cytoplasmic mislocalization of the protein, and to interfere with the availability of a co-expressed normal *FOXL2* [22]. This was corroborated by a potential promoter-specific dominant-negative effect of this polyalanine expansion [23]. However, the fact that this mutation might keep partial transactivation capacity on high-affinity promoters might explain why its phenotypic expression is often mild (i.e. BPES without POF) [23].

Most missense mutations are located in the forkhead domain. A first study by Beysen et al. [24] suggested that missense mutations in the forkhead domain leading to mislocalization and aggregation, and thus severely impairing transactivation, would lead to a more severe ovarian phenotype than missense mutations not significantly affecting protein localization and function [24]. In addition, two mutations downstream of the forkhead domain (p.S217F and p.S217C) were found to lead to a mild BPES phenotype [24]. Dipietromaria et al. [25] 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 mutations (BPES type I or type II). A clear correlation was found between the transcriptional activity of *FOXL2* mutations on two different reporter promoters and the BPES type [25]. In a very recent study by Todeschini et al. [26], 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 mutations 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 mutation. 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 [26].

FOXL2 Encompassing Deletions

Deletions of the *FOXL2* gene have been identified in 10% of the BPES patients. These patients are phenotypically indistinguishable from those carrying intragenic mutations, emphasizing that correct gene dosage of *FOXL2* is critical for normal development. The observed rearrangements range from partial and total *FOXL2* deletions to microdeletions encompassing neighboring genes. Not only are the sizes of the deletions diverse, also the breakpoint locations are heterogeneous, indicating a lack of rearrangements hotspots. Genotype-phenotype correlations might be especially helpful in providing a prognosis in newborns with BPES, mainly regarding associated features such as psychomotor retardation and POF. *FOXL2* deletions were found to lead to BPES type I. Genetic counseling and endocrinologic follow-up is therefore of utmost importance in BPES females carrying a *FOXL2* deletion [27, 28]. Other potential correlations such as an *ATR* deletion and microcephaly, and a *SOX14* deletion and limb anomalies, are still elusive [28].

Regulatory Defects

Before the identification of *FOXL2* as the disease gene for BPES, three balanced translocations were found in BPES patients. Because the deletion breakpoints were located upstream of *FOXL2*, a position effect was assumed [27, 29]. Recently, this hypothesis has been strengthened by the identification of several deletions outside the transcription unit of *FOXL2* in typical BPES patients [27, 30]. A very subtle deletion of only 7.4 kb defines the shortest region of overlap (SRO) encompassing 8 conserved non-coding sequences (CNCs) and a long noncoding RNA (lnc-RNA) named *PISRT1* that is likely co-expressed with *FOXL2* in human granulosa cells. This is in line with a presumed regulatory function of *PISRT1*, requiring a tissue and cell-type specific expression. The potential regulatory function of the CNCs was validated using in vitro luciferase assays in a *FOXL2* expressing and non-expressing cell line. Cell-type specific regulatory potential could be observed for the 3 CNCs located in the SRO. This supports that at least a fraction of the tested CNCs might be involved in the tissue-specific expression of *FOXL2*. Finally, chromosome conformation capture (3C) of a 625-kb region flanking *FOXL2* was conducted in different cellular systems. In the *FOXL2*-expressing KGN cell line, the *FOXL2* core promoter proved to come in close vicinity to 3 chromosomal fragments located upstream of *FOXL2*, of which 1 contains the 7.4-kb deletion. Furthermore, all three distant sequences were

Fig. 4. Decision tree for molecular diagnostics of BPES. Adapted from D'haene et al. [28].

found to mutually interact and contact the *FOXL2* core promoter [30].

Apart from regulatory deletions, potentially interesting sequence variations have been found in the 3' UTR of the *FOXL2* transcription unit [31, 32]. Their functional significance has not been studied so far however.

Guidelines for Molecular Genetic Testing of BPES

We proposed a decision tree for molecular genetic testing of BPES [28] (summarized in fig. 4). A first step in the diagnostic work-up of patients with a tentative diagnosis of BPES is a clinical classification of the patients into two groups: (1) typical BPES, and (2) BPES-like. Patients can be classified within the first group if: (1) the presence of four diagnostic criteria of BPES can be assessed (on a fa-

Fig. 5. Histopathological features of a GCT. The histopathological picture of a GCT shows uniform nuclei with variable grooves. HE: Adapted from Shah et al. [43].

cial picture), including blepharophimosis, ptosis, epicanthus inversus and telecanthus, or (2) at least three of the four diagnostic criteria of BPES are mentioned on a standardized clinical questionnaire. Second, a different molecular diagnostic approach is required depending on the clinical classification. In case of typical BPES, the first step is direct sequencing of the *FOXL2* open reading frame (ORF) as intragenic mutations can be detected in 72% of this group [19]. If no mutations can be detected, *FOXL2* deletion screening should be performed as a second step (e.g. using multiplex ligation-dependent probe amplification or MLPA), as gene deletions are present in 10% of this group [19]. If negative, copy number screening targeting the *FOXL2* region (e.g. using quantitative polymerase chain reaction [qPCR] or microarray-based comparative genome hybridisation [array CGH]) is recommended as a third step to exclude the presence of regulatory copy number changes affecting the long-range genetic control of *FOXL2* which have been reported in 4% of the cases [27, 30]. In case of a BPES-like phenotype, genome-wide microarray-based copy number screening is recommended, as copy number changes can be detected in a relatively large proportion of these patients (33%) [33].

***FOXL2* Impairment in Ovarian Dysfunction**

POF is defined as cessation of menses before the age of 40 for a period of at least 4 months. It is characterized by amenorrhea, hypoestrogenism and elevated serum gonadotrophin concentrations. This condition affects 1% of all women [10]. Multiple causes can underlie POF, including iatrogenic factors, autoimmune disease, metabolic

and infectious factors, genetic defects, such as X chromosome aberrations and mutations in autosomal genes. However, the cause remains unknown in the majority of POF patients. Because mutations in *FOXL2* have been associated with a syndromic form of POF, namely BPES type I, it has been considered to be a candidate gene for non-syndromic POF. Mutation studies were conducted in several cohorts with non-syndromic POF [21, 34–40]. However, only in 3 patients a variation with a presumed pathogenic effect was found. First, a heterozygous 30-bp deletion was identified in a Slovenian POF patient resulting in a polyalanine contraction of 10 alanines. Interestingly, polyalanine expansions are known to cause BPES with or without POF. The patient presented with primary amenorrhea and hypergonadotrophic hypogonadism, but was still able to conceive spontaneously and give birth to two healthy children [35]. Second, a heterozygous single nucleotide substitution (c.772T>A) leading to a non-conservative amino acid change, p.Y258N, was identified in a sporadic New Zealand POF patient. Both mutations were absent in 100 control samples [35]. Third, variant p.G187D was found in a woman with POF in the absence of BPES. While *FOXL2* localization was normal, the transactivation capacity of the mutant protein on two reporter promoters potentially relevant in an ovarian context proved to be lower than that of normal *FOXL2* [38]. These studies indicate that mutations in the *FOXL2* coding region are not a frequent cause of isolated POF.

***FOXL2* Impairment in Granulosa Cell Tumors**

Granulosa cell tumors (GCTs) represent less than 5% of all ovarian cancers. GCTs can be classified into adult and juvenile types based on different clinical and histopathologic features. Juvenile GCTs account for only 5% of all GCTs and typically occur in prepubertal girls and women younger than 30 years. These patients usually present at an early stage and have a good prognosis. On the other hand, adult GCTs occur most frequently in premenopausal women (fig. 5). Although GCTs may also present at an early stage of disease, relapses tend to occur 10–30 years after diagnosis with an estimated relapse rate of 18.6% and a mean survival rate of 5 years. Because of the tendency of a late relapse, a lifelong follow-up is advised [41]. Because no effective treatment is available to date, patients with late relapses or advanced-stage tumors at diagnosis have a poor prognosis [42]. Until recently, only little was known about the molecular basis of GCTs. Through whole-transcriptome paired-end RNA sequenc-

ing of only 4 GCTs, a somatic *FOXL2* missense mutation c.402C>G (p.C134W) was found in all of them [43]. The presence of this unique recurrent somatic mutation in more than 95% of adult GCTs has been confirmed by several follow-up studies [44–52]. Localization of the mutated protein was found to be normal [43, 44]. Differences have been found between wild-type and mutant *FOXL2* however, such as increased induction of the target aromatase by the mutation [53] and a lower capacity of the mutation to induce apoptosis, thereby compromising cell death [47, 48, 53]. A very recent study by Benayoun et al. [54] suggested that this mutation might interfere with the capacity of *FOXL2* to modulate the cell cycle.

In conclusion, the identification of this recurrent adult GCT-specific *FOXL2* mutation is a step forward for the diagnosis of adult GCTs, and might help to develop more targeted therapies and to elucidate the molecular pathogenesis of adult GCTs.

Role of *FOXL2* in the Ovary: Insights from Animal Models

Two *Foxl2* knock-out mice have been reported [55, 56]. The perinatal lethality observed in the *Foxl2* knock-out mice is high [55]. The craniofacial phenotype of the surviving homozygous animals recapitulates the BPES phenotype, with a severe eyelid malformation, and open eyes at birth [55].

Female homozygous mice are sterile with small and disorganized ovaries and lack of primary follicles [55, 56]. There are some differences between the two models however: in the first model by Schmidt et al. [55], a correct formation of primordial follicles was seen, with normal oocytes but granulosa cells that did not undergo the normal squamous to cuboidal transition; in the second model by Uda et al. [56], disorganized ovaries were seen instead of normal primordial follicles, suggesting an earlier defect. In both models, oocytes seem to be intact during the first stages of folliculogenesis and perinatally. Shortly thereafter, the follicular reserve is depleted by a massive follicular atresia leading to sterility [55, 56].

Interestingly, several findings in animal models pointed to a testis-suppressing role of *Foxl2* in the developing and adult ovary. First, this was suggested by observations in the Polled Intersex Syndrome goat. In the only natural animal model for BPES, a regulatory region upstream of goat *FOXL2* is deleted, resulting in absence of horns and sex reversal in XX animals [57]. Second, similar findings in *Foxl2*^{-/-} female mice further sustained an

‘anti-testis’ role of *Foxl2* in ovarian development [58, 59]. Indeed, *Foxl2*^{-/-} granulosa cells in mice acquire (male) Sertoli-like characteristics [59]. Third, a recent study dealing with a conditional knock-out of *Foxl2* in mice provided evidence for an ‘anti-testis’ role of *Foxl2* in the adult ovary. Indeed, loss of *Foxl2* in the adult ovary was shown to result in somatic transdifferentiation of the granulosa and thecal cells into testis-specific Sertoli- and Leydig-like cells, respectively. Moreover, this led to an immediate upregulation of testis-specific genes such as *Sox9*, assuming repression of *Sox9* by *Foxl2* [60]. The latter study profoundly changed our view on the ovary and testis as terminally differentiated organs in adult mammals. Finally, these studies might help to elucidate the molecular basis of SRY-negative XX sex reversal cases, although an initial study did not reveal any *FOXL2* mutation [60]. Finally, these findings might have potential implications for the understanding of more frequent conditions such as POF and polycystic ovary syndrome (PCOS).

FOXL2 Impairment in Other Diseases

PCOS

In the previous study, the conditional deletion of *Foxl2* in mice did not only transdifferentiate granulosa cells to testis-specific Sertoli-like cells, but also a change of thecal cells into Leydig-like cells, associated with an increase in androgen production. These findings are reminiscent of PCOS, the most common ovarian dysfunction in women in their reproductive life, as suggested by Murphy [61]. Although a link between *FOXL2* dysregulation and PCOS has not yet been demonstrated clearly in humans, constitutional *FOXL2* mutations found in syndromic POF (BPES type I) associated with androgen-induced hirsutism [61], and in a BPES patient with reported PCOS [62] might represent first hints. However, it should be noted that PCOS is very common in the general population (6–10%) and can go up to 52% in the Indian subcontinent [63, 64]. So it will be challenging to substantiate the connection between *FOXL2* disruption and PCOS in future research.

Keloid

Keloid is a dermal fibroproliferative growth caused by pathologic wound healing following skin injury, and can be defined as a hypertrophic scar growing beyond the borders of the original wound [65]. A recent genomewide association study investigating 824 individuals with ke-

loid (cases) and 3,205 unaffected controls in the Japanese population identified significant associations of keloid with four SNP loci in three chromosomal regions, one of which is 3q22.3–23 [66]. This region, with strong association with SNP rs1511412, included two genes: *LOC389151*, a hypothetical gene located 24 kb telomeric to rs1511412, and *FOXL2*, located 47 kb centromeric to rs1511412. Although speculative, a link between genetic variations influencing *FOXL2* expression and keloid susceptibility might be found in potential effects on the levels of gonadotropin-releasing hormone (GnRH) and/or steroid hormones. On the one hand, *FOXL2* is known to regulate the expression of GnRH and cholesterol metabolism and steroidogenesis, and on the other hand, it has been assumed that gonadal and steroid hormones might influence keloid formation [66].

Concluding Remarks

Mutations in *FOXL2* illustrate the concept of pleiotropy and clinical heterogeneity. More than 100 unique constitutional *FOXL2* mutations and multiple copy number changes of the *FOXL2* region, and one unique recurrent somatic mutation have been described in human dis-

ease, varying from a rare developmental condition with manifestations in the ovary and eyelid to a specific tumor in adulthood. Functional studies investigating the consequences of *FOXL2* mutations or regulatory defects considerably contributed to genotype-phenotype correlations, opening perspectives for fertility preservation. More importantly, an intact *FOXL2* function is not only crucial during development but is also needed throughout the lifetime of a female to prevent ovarian dysfunction, somatic transdifferentiation and tumor formation. In this way, researchers might be challenged by further dissection of the molecular pathogenesis of SRY-negative XX sex reversal, and more frequent conditions such as POF and PCOS. In order to provide potential targets for therapy, further insights into the regulation of expression of *FOXL2* and into potential ‘druggability’ of its downstream targets are needed. This might ultimately lead to treatment perspectives for (one of) the conditions resulting from *FOXL2* impairment.

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