ELECTRONIC LETTER

FOXL2 mutation screening in a large panel of POF patients and XX males

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Premature ovarian failure (POF) is defined as amenorrhoea for more than six months associated with raised gonadotrophins before the age of 40 years. This condition affects 1% of women in the general population. Most cases of POF are idiopathic and presumed to be genetic. Many X linked abnormalities are associated with idiopathic POF, including monosomy X in Turner syndrome and deletions and translocations implicating a number of X loci in POF.[1]

An autosomal dominant condition which is associated with POF is the blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) (MIM 110100), linked to human chromosome 3q23. In BPES type I, a complex eyelid malformation is associated with POF, while in BPES type II the eyelid defect occurs as an isolated entity.[2] The features of POF in BPES are similar to those observed in non-syndromic POF. However, the latter has not so far been linked to 3q23. Recently, Crisponi *et al*[3] have shown that mutations in the forkhead transcription factor gene *FOXL2* cause both BPES types I and II. A genotype-phenotype correlation was shown for both types of BPES; mutations predicted to result in a truncated protein lead to BPES type I, while mutations predicted to result in an extended protein cause BPES type II.[3 4] *FOXL2* is the first human autosomal gene of which dominant mutations have been shown to interfere with ovarian maintenance and POF. Expression studies have localised *FOXL2* to follicular cells in the mouse ovary, being consistent with its presumed role in follicular development and maintenance.[3] Considering the phenotypic spectrum of *FOXL2* mutations in BPES (especially type I), it was logical to expect that they might also cause other phenotypes, including non-syndromic POF.[3 5] Moreover, the causal gene is a member of the forkhead transcription factor gene family and mutations in members of this diverse gene family have been shown to cause pleiotropic phenotypes.[6]

Human XX sex reversal without SRY is a rare condition and familial cases and chromosome rearrangements are scarce.[7] However, in different domestic animals familial cases of XX sex reversal without SRY have been described, making them promising models for identifying genes that could be involved in human disorders. In 1996, the first mammalian gene involved in XX sex reversal was located through the mapping of the goat polled intersex syndrome (PIS) locus to goat chromosome 1q43.[8] PIS is a disorder showing both a dominant absence of horns affecting both sexes and autosomal recessive XX sex reversal affecting only XX animals. The PIS locus is therefore a candidate for XX maleness. Vaiman *et al*[9] proposed that goat PIS and human BPES could be encoded by a homologous gene, since the PIS locus at 1q43 proved to be the goat syntenic equivalent of the human BPES locus at 3q23 and since the PIS mutation was located in a 100 kb homologous interval of the BPES critical region.[10] While the mapping data are consistent with this hypothesis, the phenotypes are more difficult to correlate. The interspecific phenotype difference could result from a different sex determining timing between both species, from different positions of the mutations in goats and humans, or could be explained by a more dosage sensitive system in humans.[11] The involvement of *FOXL2* in BPES and its expression pattern makes it a good candidate for PIS. As a consequence, we (and others[3]) considered *FOXL2* as a candidate to be evaluated in human XX males without SRY.

MATERIALS AND METHODS

In the current study, we evaluated the presence of *FOXL2* mutations in 70 unrelated and well characterised POF cases. These women, aged from 18 to 31 years, were initially referred for secondary amenorrhoea for more than six months. All of them had had a normal pubarche and thelarche. Diagnosis of POF was confirmed by repeated high levels of FSH (>50 mUI/l) and low plasma oestradiol levels. Their karyotypes were normal (no 45,X cell line detectable in peripheral lymphocytes) and no anti-ovarian antibodies were detected (full details are available from the authors upon request). Furthermore, *FOXL2* mutation analysis was undertaken in 23 XX male/true hermaphrodite patients without SRY following the diagnostic criteria outlined by McElreavey *et al*.[7] The absence of SRY in lymphocyte DNA was shown by PCR using specific primer combinations as described by Veitia *et al*.[12] *FOXL2* screening was performed by direct sequencing of the ORF, essentially as described elsewhere.[3 4]

Key points

- The blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) is an autosomal dominant disorder in which a complex eyelid malformation is associated (BPES type I) or not (BPES type II) with premature ovarian failure (POF).
- Mutations in *FOXL2*, a forkhead transcription factor gene, have recently been shown to cause both BPES types I and II. *FOXL2* was the first human autosomal gene in which dominant mutations have been implicated in ovarian maintenance and differentiation.
- Considering that POF is part of the phenotypic spectrum of *FOXL2* mutations, it was assumed to be an interesting candidate gene for non-syndromic POF. We screened *FOXL2* in 70 unrelated POF cases in this study. Based on the synteny between the human BPES locus and the goat polled intersex syndrome (PIS) locus, we hypothesised that *FOXL2* might be an autosomal candidate gene for human XX sex reversal without SRY.
- To test this hypothesis we have sequenced *FOXL2* in 23 males lacking SRY. In both POF patients and XX males we detected seven novel *FOXL2* variations, the first ones described so far. However, no disease causing *FOXL2* mutations were found in the ORF.

Abbreviations: POF, premature ovarian failure; BPES, blepharophimosis-ptosis-epicanthus inversus syndrome; PIS, polled intersex syndrome

Table 1 Results of the mutation analysis in *FOXL2* at the genomic DNA level. The overview includes sequence variation at the genomic level and amino acid change (numbering according to Crisponi *et al*[3]), clinical information and number of subjects tested, and allele frequency of the sequence variation

Sequence variation	AA change	Clinical information	No	Allele frequency
g.738C>T	p.F167F	POF	70	8.57% (12/140), homozygous 4 cases
		XX male	23	8.71% (4/46)
		Control	55	1.82% (2/110), homozygous 1 case
		Total		6.08% (18/296)
g.759C>T	p.G174G	POF	70	0.71% (1/140)
		XX male	23	2.17% (1/46)
		Total		0.68% (2/296)
g.797G>A	p.G187D	XX male	23	2.17% (1/46)
		Total		0.34% (1/296)
g.1090C>T	p.P285S	XX male	23	2.17% (1/46)
		Total		0.34% (1/296)
g.1095T>G	p.P286P	POF	70	2.14% (3/140)
		XX male	23	6.52% (3/46)
		Total		2.03% (6/296)
g.1140G>T	p.A301A	POF	70	0.71% (1/140)
		Total		0.34% (1/296)
g.1344G>T	p.A369A	XX male	23	2.17% (1/46)
		Total		0.34% (1/296)

RESULTS

In both POF patients and XX males, we detected seven novel *FOXL2* sequence variants, the first ones described so far. These results are summarised in table 1. Five of the seven variants are silent. The two others, G187A and P285S, were found in two XX male patients as well as in unaffected family members, suggesting their non-pathogenic nature. No disease causing *FOXL2* mutations were found, suggesting that mutations in the ORF of *FOXL2* play a minor role, if any at all, in POF and XX maleness. However, we cannot exclude the existence of rare mutations whose detection would indicate increasing the sample of screened patients. Interestingly, one of the variants, 738C>T (F167F), was located at the same position as one of the pathogenic mutations (737T>A;738C>A) (F167X) reported by Crisponi *et al*.[3] This suggests that the site around position 738 (GGGGCTCTT**C**GGGGCC) might have a higher susceptibility to sequence changes.

DISCUSSION

Absence of known *FOXL2* mutations in isolated POF seems surprising at first, but it may have a plausible explanation. It has been shown that FOXL2 haploinsufficiency is a major cause of BPES (dominant negative effects have not been ruled out yet) and that the degrees of dosage sensitivity of eyelid formation and ovarian development/maintenance are different. Some mutations (amorphic/null alleles) are able to alter both processes (BPES type I), while others (thought to act as hypomorphic alleles) affect only eyelid formation (BPES type II). Thus one can safely assume that *FOXL2* mutations are most likely dominant concerning eyelid development (all documented to date), but some might be recessive concerning ovarian function (BPES type II). This type of behaviour can be understood in the light of *FOXL2* being a factor able to interact with different partners to participate in either eyelid or ovarian development. This type of interaction may account for the pleiotropy of its mutations and may lead to non-linear effects on transcriptional responses. Thus halving the amount/activity of *FOXL2* can lead to very abnormal levels of transcription (for a more detailed analysis of this phenomenon see Veitia *et al*.[13] Differences in the properties of FOXL2 complexes with eyelid or ovarian partners may explain the probable different modes of inheritance of some of its mutations. In addition, since dosage sensitivity is supposed to be higher in eyelid development, nearly all mutations of the gene are expected to generate the palpebral phenotype accompanied by POF or not, while the converse would not be biochemically likely. On the other hand, a comparison of the sequences 5′ to the *FOXL2* transcription unit (human, mouse, and goat) has allowed us to define a highly conserved region that may contain the core promoter of the gene (unpublished results). Also, regulatory mutations affecting specifically ovarian *FOXL2* expression are a plausible cause of POF and are being searched for at present in the conserved region mentioned above.

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