A novel 30 bp deletion in the FOXL2 gene in a phenotypically normal woman with primary amenorrhoea: Case report

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In a Slovene patient with primary amenorrhoea without an association with blepharophimosis/ptosis/epicanthus inversus syndrome (BPES), a novel 30 bp deletion was identified in the FOXL2 gene. We report the clinical features of this woman who has spontaneously conceived and delivered two live healthy babies. The novel deletion was predicted to remove 10 out of 14 alanines (A221_A230del), from the polyalanine tract downstream of the winged helix/forkhead domain of the FOXL2 protein. The patient’s parents and sister were shown not to carry this deletion. Despite seeing an anovulatory secretory pattern of FSH, follicles developed spontaneously. Persistent and consistent monitoring have practical implications for genetic and fertility counselling in the era when women with premature ovarian failure usually seek ovum donation. The role of FOXL2 in the development of infertility is still unclear, but several lines of evidence suggest that it plays a central role in follicle development.

Key words: fluctuating FSH/FOXL2/primary amenorrhoea

Introduction

Premature ovarian failure (POF) is defined as menopause occurring before the age of 40; it occurs in 1% of women and has genetic, autoimmune, iatrogenic or environmental origins. Genetic causes of POF probably comprise about one third to one half of all cases (Santoro, 2001). They include large chromosomal X defects, fragile X (FRAXA) and mutations in some genes: inhibin alpha (INHA), the follicle stimulating hormone receptor (FSHR) and the luteinizing hormone/choriogonadotropin receptor (LHCGR) (Aittomaki et al., 1995; Latronico et al., 1996; Beau et al., 1998; Touraine et al., 1999; Shelling et al., 2000). Patients with POF are classified into two distinct categories: patients with follicle depletion and patients with follicle dysfunction who fail to respond to gonadotropins. Interestingly, 5–10% of women conceive spontaneously after the diagnosis (Conway, 1997; Kalantaridou et al., 1998).

FOXL2 is a member of the winged helix/forkhead transcription factor family. It is known to be expressed in the human and mouse adult ovary and the developing eyelid of the mouse, with little or no expression in other tissues that have been examined (Crisponi et al., 2001). Mutations in FOXL2 have recently been associated with blepharophimosis/ptosis/epicanthus inversus syndrome (BPES), which affects eyelid development, and in the case of BPES type I, also causes POF (De Baere et al., 2001, 2003; Crisponi et al., 2001).

Seventy POF patients without eyelid defects from New Zealand and Slovenia were screened by direct sequencing of PCR products for mutations in their FOXL2 genes (Harris et al., 2002). In a Slovene patient with sporadic POF, a novel 30 bp deletion was identified that was predicted to remove 10 out of 14 alanines (A221_A230del), from the polyalanine tract downstream of the winged helix/forkhead domain of the FOXL2 protein.

We report the clinical features of this woman who has spontaneously conceived and delivered two live healthy babies.

Case report

The patient first visited the Department of Obstetrics and Gynaecology, Ljubljana, at the age of 17 because of primary amenorrhoea. She was 159 cm tall weighing 56 kg. She had a normal childhood. There was no history of mumps, diabetes, surgery or radiation to the pelvic region, or autoimmune disorders. All of her first-degree relatives had normal puberty and stature.

Her secondary sexual characteristics were well developed, including breast stage, pubic and axillary hair (P5, A4, Tanner stage 4). Physical examination revealed no signs of virilization or Turner somatic stigmata. Pelvic and ultrasound (US) examinations showed a small hypoplastic uterus and small ovaries with no visible follicles.
Initial laboratory evaluation included FSH (29.7 IU/l) (normal menopausal range 21.7–153 IU/l), LH (28.4 IU/l) (normal menopausal range 11.3–40 IU/l), E2 (0.17 nmol/l) (normal menopausal range 0–0.11 nmol/l) and prolactin (3.9 ng/ml) (normal range 4.5–40 μg/l). Thyroid evaluation (TSH, T3, T4, antithyroid antibodies), adrenal hormones and a comprehensive chemistry panel (including calcium, phosphorus, electrolytes, cholesterol, fasting glucose) were normal. Chromosome analysis showed a normal 46,XX karyotype with no evidence of mosaicism. Fifty metaphases were screened, GTG-banded chromosomes were examined at the 400–600 band level.

The patient was prescribed hormonal replacement therapy and reported normal vaginal bleeding. A repeat US visualized a 6.5 £ 4 £ 4 cm uterus, the cervix and the ovaries. After 3 years, she was switched to oral contraceptives. At the age of 22 she married and stopped the treatment by herself. Unexpectedly she became pregnant after 16 months of regular coitus without assistance of ovulation induction. The pregnancy was confirmed by US at 14 weeks of gestation. She delivered a healthy boy at term (3340 g). Six months after labour, spontaneous vaginal bleeding occurred. Thereafter, the patient became amenorrhoeic again. At her regular yearly examination, she expressed her wish to become pregnant again. The serum FSH level was 16.5 IU/l, LH 8.6 IU/l and E2 0.31 nmol/l. On vaginal US some small follicles of 5 mm in diameter were visualized in both ovaries. After fertility counselling she did not decide on in vitro fertilization treatment.

US examination was repeated every 2–4 weeks. Endocrine status showed a fluctuating serum FSH level (range 16–42 IU/l). Eight months later, US identified a developing follicle of 16 mm in diameter and serum E2 level was 0.37 nmol/l. Fourteen days later transvaginal US indicated luteinizing appearance of the follicle and thickening of the uterine endometrium to 16 mm. The woman conceived and delivered the second healthy baby girl at term (3980 g). Eleven months after delivery, spontaneous vaginal bleeding restarted. Two months later she started to use oral contraceptives.

### Materials and methods

The patient, her mother and her sister were included in the analysis. Genomic DNA was extracted from 10 ml samples of blood. The father was deceased but his genotype was determined from tracheal biopsy samples. PCR and genomic sequence analyses were performed at the Department of Obstetrics and Gynaecology, National Women’s Hospital, Auckland, New Zealand, as previously described (Harris et al., 2002). DNA was obtained from the tracheal biopsy sample using the microwave-based DNA extraction method (Banerjee et al., 1995). The fragment length analysis (Figure 1) was performed using the PCR product obtained from amplification with the FOXFRFLPF and FOXFRFLPR primers (Harris et al., 2002) to give a 197 bp fragment which was run on 10% acrylamide gel.

The study ‘Premature ovarian failure’ was approved by the National Medical Ethics Committee of the Republic of Slovenia (No. 97/05/01) and written consents were obtained from all patients and their family members.

### Results

The patient, her parents and sister were screened by PCR amplification and direct sequencing (Figure 1).
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Discussion

Premature ovarian failure has recently been associated with mutations in the FOXL2 gene (De Baere et al., 2001, 2003; Crisponi et al., 2001). Our patient has a de novo deletion within the FOXL2 polyalanine tract, and presents with primary amenorrhoea without an association with BPES syndrome.

Fluctuating FSH levels are the most characteristic hormonoal change in the menopausal transition (Burger, 1996; Lass et al., 2000). Our patient had serum FSH and LH levels fluctuating between high normal and menopausal range, and did not appear to manifest other features of an ageing ovary. Significantly, inhibin A and inhibin B were within the normal range. We did not observe a synchronized rise of FSH and LH in response to a growing follicle. In addition, despite seeing an anovulatory secretory pattern of FSH, follicles developed spontaneously. Substitution therapy with cyclic estrogen/progestagen preparations might result in a decrease in elevated gonadotropin levels and induced hormonal fluctuations with subsequent spontaneous ovulations and conception. Further work is required to determine how the abnormal gonadotropin pattern might be associated with a deletion within FOXL2.

De Baere et al. (2003) showed the existence of two mutational hotspots: 30% of FOXL2 mutations lead to polyalanine expansions, and 13% are novel out-of-frame duplications. De Baere et al. (2001) show that there is still no clear cut genotype–phenotype correlation with regard to BPES type. However, of relevance to our study, is the finding that predicted truncation before the poly A tract usually leads to type I (with POF) and expansion of the poly A, to type II (without POF) (De Baere et al., 2003). Therefore, it is perhaps not surprising that a partial deletion of the poly A tract, with the rest of the protein intact, leads to POF (with no eye defects). It may well be the poly A deletion that is responsible for the POF in at least some of the BPES type I families.

The exact role of the polyalanine tract has not yet been elucidated, although it is present in many transcription factors including some, but not all, members of the winged helix/forkhead family. The FOXL2 protein is probably involved in the inhibin/activin signalling pathway (Harris et al., 2002). The FOXL2 gene is also a good candidate for an important role in the process of follicle formation/stabilization (Schlessinger et al., 2002). It has been shown recently that the murine FOXL2 gene is essential for granulosa cell differentiation and ovary maintenance. More specifically, in these FOXL2 knockout mice granulosa cells do not complete the squamous to cuboidal transition leading to the absence of secondary follicles and oocyte atresia (Schmidt et al., 2004).

Women found to have raised FSH concentrations respond poorly to all modes of fertility treatment (Murray et al., 1999). After fertility counselling, our patient did not decide on in vitro fertilization treatment. However, hormonal production and follicular growth were monitored in the hope of detecting a spontaneous conception. Regular monitoring resulted in detection of a developing follicle of 16 mm in diameter and FSH <20IU/l. Despite the normal preovulatory E2 level, follicular growth was slower.

Persistent and consistent monitoring have practical implications for genetic and fertility counselling in the era when women with premature ovarian failure usually seek ovum donation. The role of FOXL2 in the development of infertility is still unclear, but several lines of evidence suggest that it plays a central role in follicle development. Ascertainment of the molecular basis of ovarian failure would provide a better understanding of the reproductive processes involved. It would form the basis for a genetic test for other family members at risk of developing premature ovarian failure. Aﬀected women could then make informed reproductive choices, in particular with respect to the timing of childbearing. Eventually, an understanding of the genetic defect may allow us to return fertility to these women.

Acknowledgements

Ms W. Smale is funded by the Cancer Society of New Zealand and the New Zealand Federation of Graduate Women (NZFGW). Ms S.E. Harris was a University of Auckland Postdoctoral Fellow. We would like to thank the POF patients for their involvement in this study. We would also like to thank the many clinicians who provided these patients. Funding was provided by the University of Auckland Research Committee, the Health Research Council of New Zealand and the Auckland Medical Research Foundation.

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Submitted on May 4, 2004; accepted on August 2, 2004