A new FOXL2 gene mutation in a woman with premature ovarian failure and sporadic blepharophimosis-ptosis-epicanthus inversus syndrome

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Objective: To describe a new FOXL2 gene mutation in a woman with sporadic blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) and hypergonadotropic hypogonadism.

Design: Case report.

Setting: University medical center.

Patient(s): A 28-year-old woman.

Intervention(s): Clinical evaluation, hormone assays, gene mutation research.

Main Outcome Measure(s): FOXL2 gene mutation.

Result(s): The patient with hypergonadotropic hypogonadism was diagnosed with BPES due to a new FOXL2 gene mutation.

Conclusion(s): Blepharophimosis-ptosis-epicanthus inversus syndrome is a rare disorder associated with premature ovarian failure (POF). The syndrome is an autosomal dominant trait that causes eyelid malformations and POF in affected women. Mutations in FOXL2 gene, located in chromosome 3, are related to the development of BPES with POF (BPES type I) or without POF (BPES type II). This report demonstrates a previously undescribed de novo mutation in the FOXL2 gene—a thymidine deletion, c.627delT (g.864delT)—in a woman with a sporadic case of BPES and POF. This mutation leads to truncated protein production that is related to a BPES type I phenotype. This report shows the importance of family history and genetic analysis in the evaluation of patients with POF and corroborates the relationship between mutations on the FOXL2 gene and ovarian insufficiency. (Fertil Steril® 2009;82:955-61. ©2009 by American Society for Reproductive Medicine.)

Key Words: Blepharophimosis-ptosis-epicanthus inversus syndrome, hypergonadotropic hypogonadism, premature ovarian failure, FOXL2 gene, gene mutation

Premature ovarian failure (POF) is considered in women aged <40 years with absence of menstruation (amenorrhea), hypoestrogenism, and elevated serum gonadotropins (1). More than 4 months of amenorrhea and two serum FSH levels of >40 mIU/mL obtained >1 month apart in a woman aged <40 years are the suggested criteria for diagnosing POF (2). This disorder affects 1 in 10,000 women by age 20 years, 1 in 1,000 women by age 30 years, and 1 in 100 women by age 40 years (3). In women with primary amenorrhea, the prevalence of POF is 10%–28%; in those with secondary amenorrhea, POF occurs in 4%–18% (2). Isolated and familial cases have been described; however, studies demonstrate that familial POF can vary between 4% and 35% (4–6).

Premature ovarian failure is a heterogeneous disorder determined by many pathogenic mechanisms (4). The principal causes of POF are chromosomal, genetic, autoimmune, metabolic, infectious, and iatrogenic. A large proportion of cases remain without a known cause, and these are classified as idiopathic or karyotypically normal spontaneous premature failure (7, 8).

According to some investigators, POF could be explained by two basic mechanisms, ovarian follicular depletion or follicular dysfunction. Follicular depletion can be an initial deficiency of the number of primordial follicles or an early and accelerated follicular atresia of the initial follicular endowment (2, 9).

In the last 2 decades, many studies using molecular biology technology aimed to discover a relationship between POF and genetic disorders (10–16). Among genetic causes, X-linked alterations like Turner’s syndrome, X trisomy, and X mosaicism have been reported to be responsible for a large proportion of POF cases (4). The most frequent POF-related genes in the X chromosome are the fragile site mental retardation 1 gene (FMR1) and the bone morphogenetic protein 15 gene (BMP15). However, mutations in autosomal chromosomes have been identified in many cases of POF. Mutations in genes of FSH receptor (FSHR), luteinizing hormone receptor (LHR), galactose-1-phosphatase uridyl transferase (GALT), guanine nucleotide-binding protein, α-stimulating activity polypeptide 1...
(GANS), cytochrome P450c17α (CYP17), aromatase (CYP19), carbohydrate-deficient glycoprotein (CDG), and forkhead transcription factor L2 (FOXL2) have been associated with POF (4).

Mutations in FOXL2 gene lead to blepharophimosis-ptosis-epicanthus inversus syndrome (BPES), which can be associated with eyelid malformations and POF in the same woman, according to the type of gene mutation.

This case report describes a new mutation on the FOXL2 gene in a woman with POF and sporadic BPES.

CASE REPORT

A 28-year-old nulligravida white woman was referred to a tertiary care unit for investigation of 8-year infertility. At that time the patient complained of amenorrhea since menarche at age 16 years, only having menstrual bleeding with irregular use of oral combined contraceptives.

Her gynecologic history showed normal sexual development, with breast and pubic hair compatible with Tanner stage 5, an active sex life with the same partner for 8 years, and amenorrhea for 1 year. Her medical history was positive for BPES. The family medical history for three sisters and three brothers, all phenotypically normal, was unremarkable. At the time of clinical evaluation, her physical examination showed diminished horizontal eye aperture, blepharoptosis, and epicanthus inversus. Her gynecologic examination results were within normal limits. According to a hypothesis of primary amenorrhea and primary infertility, she was submitted to a hormonal profile, transvaginal ultrasound scan, and P withdrawal test.

Her laboratory tests results were as follows: FSH 31 mIU/mL, LH 24 mIU/mL, PRL 13.9 ng/mL, TSH 2.5 mIU/mL, free T3 1.0 µIU/mL, and E2 28 pg/mL. Ultrasound scan revealed a uterine volume of 26 cm³, endometrial thickness of 4 mm, right ovarian volume of 1.1 cm³, and left ovarian volume of 1.7 cm³. The P withdrawal test results were negative. The association of hypergonadotropic hypogonadism and BPES is compatible with a diagnosis of BPES type I. Her karyotype examination showed 46,XX. The patient is currently under estrogen–progesterone replacement therapy and in a program of oocyte reception. The proband and her parents accepted an invitation to undergo genetic investigation for FOXL2 gene mutation.

METHODS

This study was approved by the internal review board, Brasília, Brazil. Peripheral blood cells were collected from the patient and her parents in an appropriate recipient with ethylenediaminetetraacetic acid.

Extraction of DNA was performed according to the “salting out” protocol. The DNA quantification was realized by electrophoresis on a 1% agarose gel with ethidium bromide. Amplification of the region of the FOXL2 gene was performed by polymerase chain reaction with four pairs of primers, as previously described by Crisponi et al. (13) and De Baere et al. (17).

The PCR products were purified and submitted to direct sequencing by the dyeoxy method using the ABI Prism BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) in the ABI Prism 377 machine (Applied Biosystems).

The obtained DNA sequences were submitted to analysis with help of software and tools like SeqScape, CodonCode Aligner, ClustalW (http://www.ebi.ac.uk/clustalw/), and Blastn (20). The sequences of the proband and her parents were compared with each other and with the reference normal sequence of FOXL2 gene.

**Results**

Throughout this analysis we detected that the proband presented a mutation in her DNA sequence of FOXL2 gene. However, her father and mother presented a normal sequence of this gene. Thus the abnormality detected in the patient is a de novo mutation leading to a sporadic case of BPES. The mutation discovered is a single-nucleotide deletion, of a thymidine base at position 864 of gene sequence (g.864delT) and 627 of exon sequence (c.627delT). Results from electropherograms suggest that this mutation is in heterozygous (Fig. 1A). The deletion observed is localized between the FOXL2 forkhead domain and the polyalanine domain. This mutation leads to a modification in reading frame and in the amino acid sequence of the predicted protein. The abnormal predicted protein presents two important characteristics: the absence of polyalanine domain and the presence of a premature termination codon leading to a 107-amino-acids-shorter protein, p.Pro209fsX61 (Fig. 1B).

**DISCUSSION**

FOXL2 is a single-exon gene that belongs to a forkhead/winged helix transcription factor family. These classes of transcription factor are involved in many of the developmental processes in humans and other organisms. The FOXL2 gene, which has 2,745 bp and its exon 1,131 bp, is extremely conserved between vertebrates (12). This gene transcribes to a 376-amino-acid protein that has a characteristic forkhead domain with 100 residues and a secondary polyalanine domain with 14 residues of alanine (12).

Mutations in FOXL2 gene have been shown to cause BPES, a rare genetic syndrome that leads to eyelid malformations with (BPES type I) or without (BPES type II) POF. Mutations that leads to truncated protein production are often related to BPES type I phenotype. However, mutations that lead to duplicated polyalanine sequence and/or elongated protein are related to BPES type II, a hypomorphic phenotype (18).

Blepharophimosis-ptosis-epicanthus inversus syndrome is an autosomal dominant inherited disease, but approximately 50% of BPES cases are sporadic, caused by a de novo mutation in FOXL2 gene. Of all described mutations, 81% are intragenic. Genomic rearrangements comprising deletions encompassing FOXL2 and deletions outside the transcription unit represent 12% and 5%, respectively. The proportion of each type of FOXL2 intragenic mutation is 11% missense mutations, 12% nonsense mutations, 44% frameshift mutations, and 33% in-frame mutations (19).

As described by De Baere et al. (20), the mutations are classified into seven groups according to their effect on the predicted protein. In groups A to D are present truncated proteins, without forkhead domain (A), with partial forkhead domain (B), with complete forkhead and without polyalanine domain (C), and with complete forkhead and polyalanine domain (D). Group E comprises frameshift mutations leading to elongated proteins with complete forkhead and polyalanine domain. Group F mutations lead to in-frame changes, and group G contains missense mutations (20).

In our case the found mutation led to a predicted protein with complete forkhead domain and without polyalanine domain (group C). The deletion of a thymidine at position 627 (c.627delT) of FOXL2 gene, between the forkhead and polyalanine domain, led to a predicted protein 107 residues shorter than the wide type and to a truncated protein (Fig. 1B). Despite its involvement in phenotype BPES determination, the specific function of the polyalanine tract is not clear. Studies with polyalanine tract expansion show an intranuclear and cytoplasmic extensive protein aggregation. In this
case the mutated protein promotes sequestration and aggregation of wide type but stays active and compensates for the sequestered protein malfunction. Therefore the related phenotype is not so severe—BPES type II (21). However, the truncated protein leads to retention of the normal protein into intranuclear aggregations, impeding its action (18). Thus the phenotype observed in this case is more expressive—BPES type I (21).

To date only 19 of the 106 described unique intragenic mutations are located between the two domains of \textit{FOXL2} gene. Of these, only three are deletions, and only two are deletions of a unique base pair (c.500delT and c.576delC). Therefore, this is the first time that the mutation c.627delT of \textit{FOXL2} gene is described in the world literature and in the \textit{FOXL2} mutation database (http://medgen.ugent.be/foxl2).

Premature diagnosis of BPES syndrome is important to enable an adequate approach and orientation of the women affected by the disease. Hormonal replacement therapy and IVF with donated oocytes have to be offered to these patients.

**REFERENCES**


