

Gene analysis in patients with premature ovarian failure or gonadal dysgenesis: A preliminary study

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Abstract

Objective: The aim of this study was to evaluate the presence of mutations in the coding region of the QM gene and fragile X in patients with premature ovarian failure and gonadal dysgenesis.

Methods: After approval by the local Ethics Committee, blood samples, in EDTA, of 100 normally ovulating women, 23 with premature ovarian failure (POF) and 14 with gonadal dysgenesis 46XX, aged less than 40 years, were screened for mutation in the QM gene coding region. All patients with POF have 46, XX karyotype and serum levels of follicle-stimulating hormone (FSH) over 30 mIU/mL. In addition, all samples from patients with premature ovarian failure underwent analysis for fragile X.

Results: The QM gene located at a hotspot region (Xq28) showed five points of mutations in a patient with premature ovarian failure. Four of them were able to change the amino acid sequence of the protein. None of our patients were diagnosed as having pre or mutant X fragile syndrome.

Conclusion: Our study suggests that Xq28 (QM gene) may be involved in ovary failure. However, further studies are needed to confirm this hypothesis.

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Keywords: Gene analysis; Premature ovarian failure; Pure gonadal dysgenesis; QM gene

1. Introduction

Premature ovarian failure (POF, OMIM 311360 and 300511) is reserved for the approximately 1% of women who experience hypergonadotropic amenorrhea before the age of 40 that accounts for about 10% of all female infertility [1]. It represents the

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end stage of a variety of disorders that result in the loss of ovarian follicles. Depending upon the age at diagnosis, the probability of a genetic, autoimmune, or idiopathic cause will be more or less likely [2]. It is important to emphasize that two functioning X chromosomes appear to be necessary for normal ovarian function [3]. In fact, several different genetic mechanisms—X chromosomal abnormalities, autosomal recessive genes causing various types of XX gonadal dysgenesis, and autosomal dominant genes can be the etiology of ovarian failure [4].

In the search for genes responsible for POF, a role for X chromosome genes was suggested by the frequent observation of X chromosome anomalies in patients [5]. Mutations in genes related to X chromosomes acting as risk factors for POF have been suggested by pedigree analysis [6], and one was identified as the premutation allele of the FMR1 gene at the FRAXA locus in Xq27 [7]. The FMR1 premutation was found in about 5% of all POF patients. Taken together, the identified genes show that POF is a very heterogeneous genetic disorder that can be inherited as a Mendelian, but more often as a multifactorial, disorder. Therefore, the search for new genes that involve the POF process is really important.

In pure gonadal dysgenesis, gonadal development and subsequent internal and external genitalia development proceed normally. However, at variable times thereafter, the gonads undergo accelerated germ cell loss, and premature degeneration and failure of the ovary ensues. It may occur in patients with both 46XX and 46XY karyotype. Females with 46XX will have a normal stature, sexual infantilism, and bilateral streak gonads. They will often present with delayed puberty and amenorrhea and will be infertile but physically responsive to estrogen treatment. The patients with 46, XX gonadal dysgenesis had decreased levels of estrogens and elevated levels of FSH and LH [8]. In addition, a mutation in an autosomal gene may be considered the etiologic factor, inherited in an autosomal-recessive fashion [9]. However, many points of pure gonadal dysgenesis etiology are unclear.

Expressive developments in both classical and molecular cytogenetics, drastically improved the ability of phenotype/karyotype relationship studies to identify structural abnormalities of the X chromo-

some and to smooth the progress of gene mapping. Indeed, Sarto et al. [10] compared karyotypes and phenotypes in women with X and found autosomal translocations and delimited a “critical region” on Xq (Xq13–Xq26) which must be intact to allow for normal ovarian development. Wyss et al. [11] reviewed 149 cases of X structural abnormalities and proposed that genes involved in ovarian development were located on the proximal part of Xp and on the distal part of Xq. Goldman et al. [12] correlated phenotypes with karyotypes in six patients with ovarian dysgenesis and X chromosomes deletions. They concluded that Xq deletions were in agreement with a relatively normal phenotype except for primary and secondary amenorrhoea and suggested that a gene located at Xq26-qter could be involved in premature ovarian failure. This alteration is called type 1 POF. Powell et al. [13] reported a patient with a secondary amenorrhoea and a balanced X: autosome translocation. They hypothesized that a second gene (type 2 POF) could be responsible for ovarian function at Xq13.3–q21.1.

In type I POF, there is some evidence for the association of fragile X syndrome and QM gene. The fragile X syndrome and its particular pattern of heredity are caused by a dynamic mutation, involving an unstable expansion of a trinucleotide (CGG) repeat at the 5' UTR of the FMR1 gene, located at Xq27.3 [14]. The QM gene, located at Xq28 and known as ribosomal protein L10 (RPL10), was originally identified as a putative suppressor of Wilms' tumor [15] besides its normal function as a ribosomal protein.

The evolutionary conservation of QM gene has revealed several interesting structural features, notably the conservation of basic and acidic domains. Basic domains have been associated with DNA binding activity and acidic, amphipathic helices with transcriptional activation, while it is clear that this gene can down-regulate c-Jun activity [16]. Due to its special localization (Xq28), conservation and function, our purpose in the present article was to screen the QM gene coding region for structural abnormalities that could be correlated with premature ovarian failure phenotype and also to evaluate the presence of mutations in the coding region of QM gene and fragile X syndrome in patients with premature ovarian failure or gonadal dysgenesis.

2. Patients and methods

2.1. Specimens

After approval by the local Ethics Committee, blood samples, in EDTA, of 100 normal ovulating women, 23 patients with premature ovarian failure and 14 with gonadal dysgenesis (46XX), aged under 40, were collected at the Gynecologic Endocrinology Sector, Department of Gynecology of the Federal University of São Paulo, Brazil. All patients with POF have karyotype 46, XX and serum levels of follicle-stimulating hormone (FSH) over 30 mIU/ml. In addition, all samples from patients with premature ovarian failure underwent analysis for fragile X syndrome by Dr Charles Schwartz at J.C. Self Research Institute of Human Genetics (Greenwood Genetic Center, South Carolina) and all of them resulted negative for this syndrome. As a control group we also screened for QM mutations a total of 100 normal women with the same average age.

2.2. RNA extraction, cDNA synthesis and SSCP analysis

RNA was extracted using the TRIZOL reagent (Invitrogen) according to the manufacturer's instructions followed by reverse transcription using the Superscript III kit (Invitrogen) in the presence of oligo-dT primers. The single-strand conformation polymorphism (SSCP) analysis was performed on PCR-amplified cDNA to screen for mutations in the QM gene coding region. The PCR reaction was performed using Mastermix (Promega) according to the manufacturer's instructions at a final volume of 50 μ l. The PCR conditions were forty 1-min cycles of denaturation at 95 °C, 1-min annealing at 60 °C and 1-min extension at 72 °C, followed by a final extension at 72 °C for 5 min. The sequences of the used primers were: sense: 5'-CGC ATT TTT GAC CTG GGG CGG-3' and antisense: 5'-CCA CAG TGC CCT GGG GCT TT-3'. Primer design, using the access number NM_006013, was conducted by using the MIT Whitehead Institute Primer3 Input located at <http://www.basic.nwu.edu/biotools/Primer3.html>. Ten microliters of the PCR product were mixed with 30 μ l stop solution (95% formamide, 10 mM NaOH,

0.25% bromophenol blue and 0.25% xylene cyanol). Samples were denatured at 95 °C for 3 min and 6 μ l were loaded onto 12.5% polyacrilamide gels (GeneGel Excel, Amersham) for SSCP analysis according to the manufacturer's instructions. Samples were run for 2 h at 431 V, 400 mA and 10 W at 10 °C in the GenePhor Eletrophoresis Unit (Amersham), gels were silver stained according to the DNA Silver Staining Kit (Amersham).

2.3. Cloning and DNA sequencing

DNA samples showing normal and abnormal migrating patterns on SSCP analysis were cloned by using the TOPO TA Cloning Kit (Invitrogen) into pCR 2.1 vector and afterwards submitted to automated DNA sequencing using the Big Dye Terminator in the ABI 3100 Genetic Analyser (PE Applied Biosystem) with M13 primers.

3. Results

3.1. SSCP and sequence analysis

Single strand conformational polymorphism (SSCP) analysis revealed that, of 23 patients with POF, the PCR product of one woman (4.3%) showed an abnormal migration pattern during electrophoresis. On the other hand none of the 100 PCR products, from women in the control group, showed a different pattern of migration during SSCP analysis. Patients with gonadal dysgenesis 46, XX did not reveal any different band patterning in polyacrilamide gels. After cloning and sequencing we were able to find five points of nucleotide changes in this sequence (Fig. 1). In four of them, alterations of the nucleotides determined changes in amino acids. Beginning from the start codon (ATG), we found in nucleotide 277 a C \rightarrow T transition where a proline was substituted for serine (pro93ser); in nucleotide 376 we found a G \rightarrow T transition where a valine was substituted for leucine (val125leu); in nucleotide 392 we found a T \rightarrow C transition where a isoleucine was substituted for threonine (ile130thr); in nucleotide 457 we found a C \rightarrow G transition where an arginine was substituted for glycine (arg153gly). Only one of them G141A proved to be a silent mutation.

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Query: ctctttcccttcggtgtgccactgaagatcctggtgtgcatgggccgcgccccgcct 122
63
      |
Sbjct: ctctttcccttcggtgtgccactgaagatcctggtgtgcatgggccgcgccccgcct 60
1

Query: gttgttaccggtattgtaagaacaagccgtaccctaaagtctcgcttctgccgaggtgtcc 182
123
      |
Sbjct: gttgttaccggtattgtaagaacaagccgtaccctaaagtctcgcttctgccgaggtgtcc 120
61

Query: ctgatgccaaagattcgcatTTTTGACCTGGGCGGAAAAAGGCAAAAGTGGATGAGTTTC 242
183
      |
Sbjct: ctgatgccaaagattcgcatTTTTGACCTGGGCGGAAAAAGGCAAAAGTGGATGAGTTTC 180
121

Query: cactttgtggccacatggtgtcagatgaatatgagcagctgtcctctgaagccctggagg 302
243
      |
Sbjct: cgctttgtggccacatggtgtcagatgaatatgagcagctgtcctctgaagccctggagg 180
181

Query: ctgccccaattttgtccaataagtacatggtaaaaagttgtggcaaaagatggcttcata 362
303
      |
Sbjct: ctgccccaattttgtccaataagtacatggtaaaaagttgtggcaaaagatggcttcata 300
241

Query: tcgggtgcggtctccactccttccacgtcatccgcacaaacaagatgttgctctgtgctg 422
363
      |
Sbjct: tcgggtgcggtctccactccttccacgtcatccgcacaaacaagatgttgctctgtgctg 300
301

Query: gggctgacaggctccaacaggcatgcgagggtgcctttgaaagcccagggcactttgg 482
423
      |
Sbjct: gggctgacaggctccaacaggcatgcgagggtgcctttgaaagcccagggcactttgg 420
361

Query: ccagggttcacactggccaagttatcatgtccatccgcaccaagcctgcagaacaagg 542
483
      |
Sbjct: ccagggttcacactggccaagttatcatgtccatccgcaccaagcctgcagaacaagg 420
421

Query: agcatgtgattgaggcccctgggcaggccaagtcaagtttctggccgccagaagat 602
543
      |
Sbjct: agcatgtgattgaggcccctgggcaggccaagtcaagtttctggccgccagaagat 535
478

Query: ccacattctcaaaaaagnggggcttcaccaagttcaatgctgatgaatttcgaagaca 662
603
      |
Sbjct: ccaca-tctcaaaagaagtgggg-cttcaccaagttcaatgctgatgaattt-gaagaca 591
536

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Fig. 1. The mutated QM region in the gene sequence. The rectangles (1–5) are indicating the mutation points found in a patient with premature ovarian failure. The start codon ATG is underlined at the top of the sequence.

3.2. *X fragile syndrome analysis*

None of our patients were diagnosed as having pre or mutant X fragile syndrome.

4. Discussion

We were able to identify structural abnormalities in the QM gene, also known as Ribosomal Protein L10 (RPL10), the coding region of one patient suffering from premature ovarian failure. The gene, located at a hotspot region (Xq28) containing genes involved in normal ovary development, showed five points of mutations. Four of them were able to change the amino acid sequence of the protein. To the best of our knowledge this is the first time that structural abnormalities in QM gene were found in patients with premature ovarian failure. It is also very important to mention the weak points of our study. Among them we are aware that we only included 23 patients with POF and 14 with gonadal dysgenesis. However, the fact that we could not observe any polymorphisms and/or mutations in the 100 patients of the control group makes our results at least very interesting. Indeed, due to the extreme conservation of QM gene (RPL 10), of the order of 1% change every 22 million years, is very unlikely to find mutations in this gene [17].

Regarding the found mutations, it is important to emphasize that the one which occurred in the amino acid at position 125 of QM protein is located, exactly, in very conserved regions. Indeed, scanning these regions of human QM against Prosite, we found two putative protein kinase C (PKC) phosphorylation sites, SiR and SkK, through which PKC may introduce phosphate into QM and therefore regulates its binding to c-Jun that is important for ovarian function [16].

It is also important to emphasize that we did not find the presence of the fragile X syndrome in our patients, very likely, due to the small number of studied patients. Indeed, mutation of genes related to patients with this syndrome have been identified in 0.8–7.5% of women with sporadic premature ovarian failure and in up to 13% of women with familial premature ovarian failure [18–21]. One explanation might be the occurrence of alterations in autosomal genes, such as FSHR, LHR, FOXL2, INHA, GALT or related to oocyte growth, such as mutation in bone morpho-

genetic protein-15 that might be associated with POF [22,23].

We here hypothesize that due to the fact that QM (RPL10) is considered to be a putative tumor suppressor gene and also that the conformational change observed in the mutated protein is compatible with an increase in protein stability, one could speculate that these mutations might lead to uncontrolled activation of QM in ovary cells with a consequent increase in the occurrence of apoptosis. Our study further suggested that Xq28 may be involved in ovary function, but further research is necessary to confirm the role of QM in ovary failure.

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