Xq;autosome translocation in POF: Xq27.2 deletion resulting in haploinsufficiency for SPANX

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Introduction

Premature ovarian failure (POF), also called primary ovarian insufficiency (POI), is defined as irregular menses in women younger than 40 years with menopausal-level serum gonadotropins [1]. Spontaneous onset POF may result from numeric and structural chromosomal abnormalities, fragile X premutations, autoimmune disorders and rare syndromes, though in most cases the etiology remains unknown [2].

Structural chromosomal abnormalities, such as deletions and translocations of specific regions on the X chromosome, have been associated with POF. This association may be due to the haploinsufficiency of genes necessary for normal ovarian function. Standard karyotypes and deletion mapping have identified three regions of interest for POI,

one in Xp and two in X (POF1: X26-28 and POF2: X13.3-22) [3, 4]. Several candidate genes in X have been proposed by mapping genes interrupted by the breakpoints in X;autosome translocations. These candidate genes include *DIAPH2*, *XPNEP2*, *DACH2*, *POF1B*, *CHM* and *NXF5*, though none of these genes have a well-defined role in POF [5]. Introduction of high-resolution microarray CGH has refined the ability to detect cryptic chromosomal deletions and duplications in the X POF regions, potentially facilitating identification of candidate genes [6].

We present a patient with infertility and primary ovarian insufficiency having a balanced Xq;autosome translocation near the POF2 region, with a cryptic deletion at Xq27.2, identified with array CGH and confirmed by fluorescent in situ hybridization (FISH). The deletion corresponds to the loci for SPANX (sperm protein associated with the nucleus, X-linked), which play roles in spermatogenesis and may represent candidate genes for POF.

Capsule A case of POF with an Xq;autosome translocation near the POF2 region and a cryptic deletion in the POF1 resulting in haploinsufficiency for SPANX.

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Materials and methods

Patient history

23-year-old woman presents with irregular menses and infertility. She reported normal pubertal timing and progression, with menarche at age 13 years. At age 15 years, she was placed on oral contraceptive pills for cycle regulation. After discontinuation of oral contraceptive pills at age 22, she experienced oligomenorrhea and infertility. She denies galactorrhea, hirsutism, acne, hot flushes or vaginal dryness. She denies a history of mumps oophoritis. There are no female siblings. No relatives are said to have shown infertility or early menopause, autoimmune disorders, developmental delay or Parkinson's disease. Her height is 157.5 cm and weight is 90 kg. There is no

evidence of deafness, high arched palate, webbing of the neck, thyromegaly, hyperpigmentation, vitiligo, or other somatic anomalies. Pelvic examination revealed normal female external genitalia, a small anteverted uterus and no adnexal masses. Pelvic ultrasound showed a normal sized uterus and scant antral follicles.

Baseline laboratory evaluation included day 3 FSH 61.2 mIU/ml, estradiol <20 pg/ml. Repeated on day 3 of a subsequent cycle, FSH was 21.6 mIU/ml, and estradiol <20 pg/ml. Values were normal for TSH, prolactin and AM cortisol, anti-thyroid antibodies, anti-adrenal antibodies and FMR1 pre-mutation screening.

High-resolution karyotype

High-resolution chromosome analysis and GTG banding of cultured peripheral blood lymphocytes were performed according to standard protocol [7]. Chromosomes were analyzed at 550–600 band resolution.

Array comparative genomic hybridization

Genomic DNA was isolated from the patient's peripheral blood by standard methods. Oligonucleotide-based microarray analysis was performed using the NimbleGen CGX-3 array, which provides 135 k oligonucleotide coverage. This whole-genome microarray is designed by Signature Genomic Laboratories (Spokane, WA) and manufactured by Roche NimbleGen (Indianapolis, IN). Genomic DNA was labeled with Cy3 or Cy5 using a NimbleGen Dual-Color DNA

Labeling Kit, and sex-matched controls (Promega, Madison, WI) were used. Array hybridization and washing were performed as specified by the manufacturer (Roche NimbleGen). Arrays were scanned on an Axon Genepix 4000B scanner (Molecular Devices, Sunnyvale, CA) and analyzed using NimbleScan software. Results were displayed using web-based data visualization software (Genoglyphix®; Signature Genomic Laboratories).

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was performed according to the manufacturer's protocol, using a labeled BAC probe (RP11-1082H4) obtained from Empire Genomics (Buffalo, NY).

Results

High-resolution karyotype

High resolution karyotype analysis revealed an apparently balanced translocation: 46,X,t(X;1)(q22.1;q42.1), with no apparent macroscopic loss of genetic material from the translocated chromosomes (Fig. 1).

Array comparative genomic hybridization

Array CGH revealed a cryptic 382–482 kb deletion at Xq27.2 (arr Xq27.2(140,201,306–140,583,606)x1) (Fig. 2).

Fig. 1 Standard GTG karyotype demonstrating the translocation 46,X,t(X;1)(q22.1;q42.1)

Fig. 2 Microarray comparative genomic hybridization (Signature Chip OS, resolution 10 kb) revealing a cryptic 383–484 kb deletion at Xq27.2, (arr Xq27.2 (140,201,306-140,583,606)x1)

Based on the Genoglyphix®, Hapmap and UCSC genome browser database, analyses, the deletion corresponds to the loci for the genes *SPANXA1* and *SPANXA2* (sperm protein associated with the nucleus, X-linked). The Ensembl database analyses suggest that the deletion spans *SPANXC*, as well as *LDOC1* (leucine zipper, down regulated in cancer 1), 1 RNA gene (*U6.560*) and 3 pseudogenes (*RP3-50715.1*, *GS1-821.3*, *GS1-164 F24.1*) and that *SPANXA1* and *SPANXA2* are adjacent to the deletion.

Fluorescence in situ hybridization

In order to confirm the microarray findings and localize the deletion, FISH analysis was performed with a BAC probe (RP11-1082H4) mapping to the deleted region (Fig. 3). The deletion was present on the X chromosome that was not involved in the translocation.

Fig. 3 Fluorescent in situ hybridization (FISH) using bacterial artificial chromosome (BAC) probe (RP11-1082H4) localized and confirmed the deletion given the absent pink fluorophore on the X chromosome not involved in the translocation

Discussion

Our patient exhibits a balanced Xq;autosome translocation near the POF2 region, with a cryptic 382–482 kb deletion at Xq27.2. The deletion we observe falls within the region connoted by POF1, one of several X-regions for which gene specifications (POF1, POF2, POF3) have been proposed. However for none are actual loci defined. The deletion encompasses the loci for *SPANX* (sperm protein associated with the nucleus, X-linked). *SPANX* plays a role in spermatogenesis and could play a role in folliculogenesis, representing a candidate gene for POF.

Parental array studies to determine if the deletion is sporadic or familial are unfortunately not available; thus, a benign polymorphism cannot unequivocally be excluded. In addition, X-inactivation studies could determine if the X chromosome carrying the microdeletion is activated, but this would not be expected in an X;autosome translocation. Our patient's clinical presentation and findings are consistent with those of women with X deletions who experience oligomenorrhea, followed by secondary amenorrhea and premature ovarian failure [8]. The deleted region we detected contains 2–6 annotated genes of which those most plausibly related to POF are *SPANXA1*, *SPANXA2* and *SPANXC*. These genes play a role in spermatogenesis and plausibly may represent candidate genes for POF.

X-autosomal translocations have long been known to be associated with POF, possibly due to aberrations in pairing, haploinsufficiency of X-linked genes or X inactivation during folliculogenesis [9]. Alternatively, Rizzolio et al. have proposed that autosomal genes expressed in the oocyte could be downregulated by epigenetic mechanisms when translocated next to a POF critical region on the active X chromosome. It is also possible that a positional effect involving the POF 2 critical region could alter the expression of the translocated autosomal genes, resulting in our patient's phenotype.

SPANX genes encode testis-specific proteins, which localize to the nuclear envelope of post-meiotic spermatids

[10]. SPANX mRNA is rarely detected in spermatogonia and spermatocytes [11]. The temporal expression of SPANX proteins suggests a role in spermatogenesis. There have been no reports examining the presence of SPANX mRNA or proteins in oocytes. This case report of POF in the setting of a deletion involving the loci for *SPANXA1*, *SPANXA2* and *SPANXC* may suggest an analogous role in folliculogenesis, with SPANX as a possible candidate gene family for POF. Molecular studies to detect perturbations are needed, as well as functional studies involving SPANX expression in oocytes.

Conflict of interest None of the authors have a conflict of interest to report.

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