CASE REPORT

Molecular cytogenetic definition of a translocation t(X;15) associated with premature ovarian failure

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Objective: To characterize the breakpoints of a t(X;15) found in a woman with premature ovarian failure (POF).
Design: Case report.
Setting: Molecular and cytogenetics unit in a university-affiliated hospital.
Patient(s): A 19-year-old infertile woman presenting with a normal female phenotype but primary amenorrhea.
Intervention(s): Molecular cytogenetic analyses and genetic counseling.
Main Outcome Measure(s): Translocation t(X;15) defined by fluorescence in situ hybridization (FISH) and array comparative genomic hybridization (array CGH).
Result(s): Chromosome and FISH analysis revealed 46,XX, t(X;15)(Xq22.1;p11); the active X was translocated and had been inherited from her mother. Detailed molecular characterization by FISH showed that the *NXF5* (nuclear RNA export factor 5) gene was contained in the clone spanning the breakpoint on the X chromosome.
Conclusion(s): The *NXF5* gene is an appealing candidate for POF because it shows functional homology with the *FMR1* (fragile X mental retardation 1) gene. Further analyses of its expression as well as mutation screening in other POF patients will help to elucidate its role. (Fertil Steril® 2010;94:1097.e5–e8. ©2010 by American Society for Reproductive Medicine.)

Key Words: Array CGH, chromosome aberration, FISH, infertility, lymphocytic karyotype, premature ovarian failure, X autosome translocations

Premature ovarian failure (POF, OMIM 311360 and 300511), a disorder characterized by amenorrhea and elevated serum gonadotropin levels before 40 years of age, shows a heterogeneous etiology that includes environmental, autoimmune, and genetic factors. The X chromosome plays a primary role in this pathology: the X structural abnormalities (i.e., large deletions, inversions, and balanced X;autosome translocations) are often associated with POF. Cytogenetic and molecular analysis of X rearrangements have highlighted that a large region from Xq13.3 to Xq26/27, called the "critical region," is essential for normal ovarian function and a normal reproductive lifespan (1, 2).

Initially, it was suggested that genes for ovary development and/or function could be clustered in this region and their haploinsufficiency was responsible for this pathology; however, it is now evident that the etiology of POF is more complex, and epigenetic phenomena have been put forward (3). We present the case of a patient with primary amenorrhea associated with t(X;15)(Xq22.1;p11). A detailed molecular characterization of the breakpoints, performed to gain further insight into etiology of our patient's POF, revealed that the *NXF5* gene was contained in the clone spanning the breakpoint. Its function and expression are discussed in relation to its putative role in this condition.

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MATERIALS AND METHODS

Patient

The proband, a 19-year-old woman, was born at term after an uneventful pregnancy and delivery from healthy, nonconsanguineous parents. Her birth weight was 3,000 g, length 50 cm, and her growth and developmental milestones were normal. At 6 years of age, she presented premature pubarche. She came into our observation at the age of 14.3 years for puberal delay. Her height (161 cm) was in the 50th percentile, and her weight (64 kg) was in the 75th to 90th percentile; she did not present any major dysmorphism.

Her breast development was classified as Tanner stage B2, corresponding to the age of 11.2 ± 2.2 years, and pubic hair development was classified as stage PH3, corresponding to the age of 12.4 ± 2.2 years. Pelvic ultrasound examination revealed small ovaries with some cortical cysts. Her hormone profile was normal for thyroid-stimulating hormone (TSH), free thyroxin (FT_4), free triiodothyronine (FT_3), prolactin, cortisol, 17α-hydroxyprogesterone (17-OHP), androstenedione, and testosterone; her levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were high (49.0 and 138 mIU/mL, respectively), typical of menopause. She had primary amenorrhea with no menarche and no progression of secondary sexual

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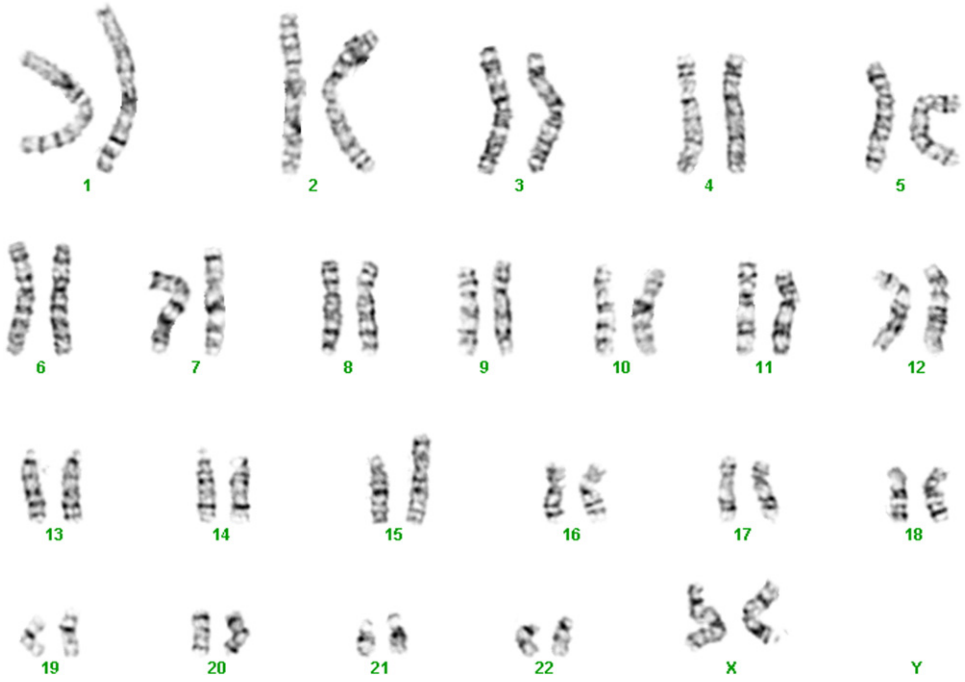
FIGURE 1

G-banding karyotype showing the translocation t(X;15).



Bertini. A t(X;15) translocation associated with POF. Fertil Steril 2010.

characteristics at 16 years of age. She had normal menses after therapy with estrogen and a low-dose combined estrogen/progesterone preparation.

Cytogenetic and Fluorescence in Situ Hybridization Investigations

We analyzed G-banded and Q-banded chromosomes of the patient's cultured peripheral blood lymphocytes. Chromosomal preparations were obtained according to standard techniques. We also performed further fine mapping of the breakpoints by fluorescence in situ hybridization (FISH) with intervening tiling clones. The PACs and BACs were obtained from Resources for Molecular Cytogenetics (http://www.biologia.uniba.it/rmc/index.html), C.H.O.R.I.(http://bacpac.chori.org), and the YAC Screening Center (http://www.sanraffaele.org).

X Inactivation

The pattern of X inactivation was determined using the HUMARA (human androgen receptor) assay of Allen et al. (4). Briefly, genomic DNA was incubated overnight with *Hpa*II (8 IU/mg DNA). Amplified products of the *AR* gene were obtained from both digested and undigested samples from females, and subjected to 9% nondenaturing polyacrylamide gel electrophoresis. Allele bands were detected on silver-stained gel. In male family members, the HUMARA haplotype was determined on polymerase chain reaction (PCR) products from undigested DNA.

Array Comparative Genomic Hybridization

Genomic DNA from the patient was isolated from peripheral blood by standard methods; a normal control female DNA was obtained from Promega (Madison, WI), and 1 μg of genomic DNA both from the patient (test sample) and from the control (reference sample) was digested with *RSA*I and *ALU*I restriction enzymes. Test and reference DNA were differentially labeled with Cy5-dCTP or with Cy3-dCTP using random prime labeling according to the manufacturer's protocol (Agilent, Santa Clara, CA). The labeling reactions were applied to the 44 K arrays and incubated in a oven for 24 hours at 65°C. Slides were washed and scanned using the Agilent scanner. Identification of individual spots on scanned arrays was performed with the Agilent dedicated software, as was the filtration, normalization, and exclusion of spots with aberrant morphology or high background.

RESULTS

Cytogenetic and FISH

Standard cytogenetic investigations in the proband showed a de novo translocation, 46,XX, t(X;15)(Xq22;p11), in all 50 metaphases analyzed (Fig. 1). The breakpoint on chromosome 15 was localized on the p arm; the FISH with D15Z4 (p11.1-q11.1) probe showed signals on the centromere of chromosome 15 and the derivative 15, whereas the D15Z1 (p11.2) probe revealed signals on the derivative X and on 15p11.2.

Fine localization of the breakpoint on the chromosome Xq was defined by FISH by use of a cluster of PAC and BAC clones at Xq21.33-Xq22.2 (Fig. 2). The BAC clone CTD-2006G2, located