Disruption of HDX gene in premature ovarian failure

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We present a case of a 19-year-old phenotypically normal girl with premature ovarian failure. Cytogenetic analysis using G banding and fluorescence in situ hybridization (FISH) from cultured peripheral blood lymphocytes of the patient and the family revealed a de novo X;15 translocation and the imbalance to be 46,X,t(X;15)(Xpter→Xq21::15q11→15qter;15pter→15q11::Xq21→Xqter).

ish (CEPX+, wep15+, ISNRPN+, PML+, D15S10+, wcp15-, SNRRN-, PML-)[20]. The X chromosome inactivation (XCI) assay revealed a completely skewed XCI pattern in which selective pressure favors an active maternal allele. The Affymetrix 2.7 M cytogenetics whole-Genome array confirmed the chromosomal imbalance and identified disruption of the HDX gene at Xq21, the translocation breakpoint.

Keywords HDX gene, premature ovarian failure, X chromosome inactivation, X;autosome translocation

Abbreviations FISH: fluorescence in situ hybridization; XCI: X chromosome inactivation; POI: premature ovarian insufficiency; USG: ultrasonography; FSH: follicle-stimulating hormone; LH: luteinizing hormone; TSH: thyroid-stimulating hormone; HDX: highly divergent homebox; AR: androgen-receptor; CrR: corrected ratio.

Introduction

Premature ovarian failure (POF; POF1: MIM 311360, POF2: MIM 300511; POF3: MIM 608996) [Beck-Peccoz and Perzani 2006], premature ovarian insufficiency (POI), are characterized by absences of menstruation (primary amenorrhea) or premature depletion of ovarian follicles with elevated gonadotrophin and low estrogen before the age of 40 years (secondary amenorrhea) [Cordts et al. 2011]. Iatrogenic intervention, autoimmune diseases, infections, X chromosome defects, and monogenic defects are possible causes of POF [Beck-Peccoz and Perzani 2006]. There are different types of X chromosome aberrations. These include monosomy X, trisomy X, partial deletions, and X; autosome translocations. X;autosome translocations are rare chromosomal abnormalities with an incidence rate of 1 in 30,000 in live births [Speroff and Fritz 2005].

Premature ovarian failure is clearly a heterogeneous disorder associated with mutations in FMR1 [POF1, (OMIM: 311360)] on Xq27.3, DIAPH2 [POF2A, OMIM: 300511] on Xq21.33 and POF1B [POF2B, (OMIM: 300604) on Xq21.1, FOXL2 [POF3, (608996)] on 3q22.1, BMP15 [POF4, (OMIM:300510)] on Xp11.22, NOBOX [POF5, (OMIM:611548)] on 7q35, NIGLA [POF6, (OMIM:612310)] on 2p13.3, and NRSAI [POF7, (OMIM:612964)] on 9q33.3. In this paper, we report a phenotypically normal girl with an X;15 translocation that interrupts the highly divergent homeobox (HDX) (RefSeq accession number: NG_017158.1) gene on Xq21.1.

Case Report

A 17-year-old girl with secondary amenorrhea was admitted to the Department of Medical Biology, Section of Medical Genetics at Ondokuz Mayis University. She was the fourth child of healthy non-consanguineous parents. Her body height was 158 cm, and her weight was 51 kg. She menstruated at age of 12 and then at 17 years of age with continued hormone therapy. Ultrasonography (USG) revealed small ovaries with arrested folliculogenisis. Laboratory investigations demonstrated that her hormonal levels were as follows: follicle-stimulating hormone (FSH), 75.6 mU/mL; luteinizing hormone (LH), 100.5 mIU/mL; prolactin, 16.94 ng/mL, and estradiol, 83 pg/mL. FSH and LH were higher than normal. The measurement of thyroid-stimulating hormone (TSH) levels was normal.

Results and Discussion

G banding cytogenetic analysis from cultured peripheral blood lymphocytes of the proband revealed a karyotype:
Chromosomal imbalance was identified on the X chromosome. The cytogenetic study of the patient revealed a de novo balanced translocation t(X;15) (q21;q11). The FISH analysis confirmed the presence of the translocation breakpoint involving Xp22 and 15q11.

The translocation breakpoint falls within the Xq21.2 region, which contains several genes associated with POF. Among these, the HDX gene is of particular interest, as it is involved in oogenesis and ovulation. The disruption of HDX gene at the translocation breakpoint could be responsible for the disruption of oogenesis and ovulation in the patient.

Cytogenetic studies have suggested that the long arm of the X chromosome is mainly involved in defects of ovulation. The disruption of HDX gene at the translocation breakpoint could be responsible for the POF phenotype observed in the patient. Further studies are needed to confirm this hypothesis.

In conclusion, the present study reports a patient with POF and a de novo balanced translocation t(X;15) (q21;q11). The cytogenetic and FISH analysis confirmed the presence of the translocation breakpoint involving Xp22 and 15q11. The disruption of HDX gene at the translocation breakpoint could be responsible for the POF phenotype observed in the patient.
of germ cells from the pool [Schlessinger et al. 2002]. Second, translocation of regulatory domains to another position on the genome might cause alternations in transcriptional regulation due to "position effect" [Persani et al. 2009]. Lastly, in female balanced X;autosome translocation carriers, the normal X chromosome is usually inactivated allowing full expression of genes on the translocated segments [Schmidt and Du Sart 1992; Yang et al. 2011]. Female balanced X;autosome translocation carriers are generally phenotypically normal because the normal X chromosome is completely inactivated in these patients as a result of phenotypic plasticity to prevent deleterious monosomy. Similarly, as reported here a balanced X;15 translocation has a completely skewed XCI pattern in which the selective pressure for XCI should be against the translocated autosomal material in order to prevent deleterious monosomy.

In this study, we directly demonstrated that X;15 translocation interrupts the Xq21 HDX gene in a phenotypically normal female with POF. The findings and our observations suggested that HDX could be critically important in the development of ovarian follicles or the regulation of ovarian gene expression and alterations of this gene may lead to premature ovarian failure. Further characterization and analysis of the genomic region encompassing HDX, in POF patients, as well as its effects on RNA and protein levels in patients should provide information for ovarian failure.

**Materials and Methods**

Prior to the study, the patient and her family was informed and gave signed informed consent to participate in this study. All protocols involving the use of human tissue specimens were approved by the Institutional Review Board.

**Cytogenetic analysis**

Chromosomal analysis was performed from peripheral blood samples using standard procedures. G banding analysis was performed on metaphases obtained from PHA stimulated lymphocytes from the proband and all of her family members according to standard procedures.

**FISH analysis**

FISH was performed on lymphocyte metaphase spreads of the patient using the CEP X SpectrumOrange/Y SpectrumGreen Direct Labeled Fluorescent DNA Probe Kit (Abbott Molecular 07)20-050, Des Plaines, IL) and Whole Chromosome Painting probe 15 (Cytocell, Aquarius®LPP15 G9X, UK).

**Cytogenetics array analysis**

Molecular cytogenetic array analysis was performed in DNA sample obtained from the patient, according to the manufacturer’s protocol for Affymetrix GeneChip® Cytogenetics 2.7M Whole-Genome Array (Affymetrix Inc. Santa Clara, CA, USA) with average genome coverage of approximately one
marker per 735 bp. The array consisted of approximately 2.7 million markers, including 2,361,876 non-polymorphic markers and 400,103 single nucleotide polymorphic markers for unbiased coverage of the genome. The microarray image data was extracted using the Command Console Software 3.0.1 (AGCC) (Affymetrix Inc.). Data were analyzed using Chromosome Analysis Suite Software v1.0 (ChAS) (Affymetrix Inc.) with a threshold SNP QC using Chromosome Analysis Suite Software v1.0. Duplication or loss of chromosome number at given loci was considered significant only if the duplication or loss of the chromosome exceeded 100 kb. The genomic locations were retrieved from the National Center for Biotechnology Information (NCBI) build 28 (hg 18). The gene located at the translocated X chromosome region is HDX gene and on chromosome 15 includes: LOC727924, CXADRP2, LOC2837.

**X-chromosome inactivation analysis**

DNA was extracted from 200 µl venous blood sample by NucleoSpin Blood kit as in the protocol supplied by the manufacturer (Macherey-Nagel, Düren, GERMANY) and evaluated for quality and concentration using ND-1000 spectrophotometer (Nanodrop, Wilmington, USA) and agarose gel electrophoresis.

Genotyping of the highly polymorphic CAG repeat in the androgen-receptor (AR) was performed to assess the XCI pattern [Allen et al. 1992]. The DNA was divided into two identical aliquots, one of which was incubated overnight at 37°C with methylation-sensitive restriction enzyme HpaII (MBI Fermentas, Vilnius, Lithuania) for the digestion of unmethylated (or active) alleles. A second restriction enzyme, Rsal (MBI Fermentas), which recognizes a four base pair sequence not present in the amplified region of the AR locus was also included in the reaction to facilitate the HpaII digestion process. The other aliquot of the DNA was digested with Rsal alone as a control. Male DNA with cytogenetically verified 46,XY karyotype was used as a control for incomplete digestion. After restriction enzyme digest, residual DNA was amplified by using the primers 5'-GTC CAA GAC CTA CCG AGG AG-3' and 5'-CCA GGA CCA GTG AGC CTG TG-3'. PCR products were separated on 8% denaturing 29:1 acrylamide/bisacrylamide gel for 3 h at 15W. Densitometric analysis of the alleles was performed at least twice for each sample using the appropriate software (MultiAnalyst version 1.1; Bio-Rad, Hercules, California). Since the number of cycles could be critically important for the outcome of densitometric analyses, samples were subjected to 30 cycles of amplification during PCR. Products were visualized by ethidium bromide staining, and densitometric analysis of the alleles was performed at least twice for the MultiAnalyst version 1.1 sofware. A corrected ratio (CrR) was calculated by dividing the ratio of the predigested sample (upper/lower allele) by the ratio of the non-predigested sample for normalization of the ratios that were obtained from the densitometric analyses. The use of CrR compensates for preferential amplification of the shorter allele when the number of PCR cycles increases. A skewed population is defined as a cell population with greater than 80% expression of one of the AR alleles. This corresponds to CrR values of < 0.33 or > 3.

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**Author contributions:** Cytogenetic analysis and editing of manuscript: GO; Preparation of the manuscript and cytogenetic analysis: SG; XCA and preparation of related parts in manuscript: OEO; FISH analysis: AT; XCA and editing of manuscript:TO; Clinical evaluation of the patient: IK.

**References**


