CLINICAL REPORT

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Premature Ovarian Failure, Absence of Pubic and Axillary Hair With De Novo 46,X,t(X;15)(q24;q26.3)

Claudio Giacomozzi,[1] Francesca Gullotta,[2] Giovanni Federico,[3] Isabella Colapietro,[2] Anna Maria Nardone,[2] and Stefano Cianfarani[1]*

[1]Molecular Endocrinology Unit-D.P.U.O., Bambino Gesù Children's Hospital-"Rina Balducci" Center of Pediatric Endocrinology, Tor Vergata University, Rome, Italy

[2]Laboratory of Medical Genetics, Fondazione PTV-Policlinico Tor Vergata, Rome, Italy

[3]Unit of Endocrinology and Diabetes, Department of Pediatrics, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy

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We report on an adolescent girl with premature ovarian failure (POF), de novo unbalanced translocation X;15(q24;q26.3) with partial Xq24 duplication, and absence of pubic and axillary hair. Endocrine assessment showed normal adrenal and ovarian function. Chromosomal abnormality was identified by standard cytogenetic methods, array-CGH, and FISH analysis. Mutation analysis showed normal androgen receptor genes. Pubic and axillary hair began developing during estrogen + progesterone therapy. Our patient demonstrates that a distal X-breakpoint involving POF1 locus is able to cause POF without virilization during adolescence. © 2010 Wiley-Liss, Inc.

Key words: secondary amenorrhea; premature ovarian failure; pubarche; translocation X ;15 (q24 ;q26.3)

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INTRODUCTION

Premature ovarian failure (POF [MIM 311360]) refers to primary or secondary amenorrhea before the age of 40 years. In women with POF there is anovulation, infertility, and reduced estrogen levels which result in major health problems. Approximately 1% of women is affected with POF by the age of 40 years, whereas only 0.1% is affected by the age of 30 [Coulam et al., 1986; Luborsky et al., 2003]. The diagnosis is based on clinical presentation and the finding of repeatedly elevated FSH levels [Conway, 2000]. The causes of POF are heterogeneous and the acquired forms may occur in the context of autoimmune disease, infections, or following anti-cancer treatment [Conway et al., 1996; Howell and Shalet, 1998]. A genetic contribution is suggested by the occurrence of families with many affected women. POF is heritable in up to 30% of patients and is genetically heterogeneous [Coulam et al., 1986; Luborsky et al., 2003]. The chromosome abnormalities described in POF include either X chromosome or autosome alterations. Partial or complete monosomies for chromosome X and X;autosome translocations are well-documented causes of primary amenorrhea and ovarian dysfunction. Many of these rearrangements are located in the region Xq13-q26 suggesting the existence of a POF "critical region" [Therman et al., 1990; Zinn, 2001; Rizzolio et al., 2006]. Other authors have identified two specific regions on Xq defined as POF loci: POF1 Xq26-qter [Tharapel et al., 1993] and POF2 Xq13.3-q21.1 [Powell et al., 1994]. Distal deletions involving the POF1 region result in POF at age 24–39 years [Tharapel et al., 1993], whereas translocation involving the POF2 region causes POF at an earlier age [16–21 years, Powell et al., 1994]. It has been suggested that one or multiple genes located in this region are required in double dose for normal ovarian function. Several different translocations involving an autosome and the X chromosome have been described, each involving a different chromosomal breaking point [Rizzolio et al., 2006]. Clinical presentation is variable in each case, but, to date, absence of pubic and axillary hair associated with normal puberty and secondary amenorrhea has not been reported.

PATIENTS AND METHODS

Patient

A $15^{9}/_{12}$-year-old girl presented with secondary amenorrhea. She was the first daughter of healthy parents (a Japanese man and a Peruvian woman with menarche at the age of 11 years). She was

Claudio Giacomozzi and Francesca Gullotta contributed equally to this work.

*Correspondence to:
Stefano Cianfarani, M.D., Department of Public Health and Cell Biology, "Rina Balducci" Center of Pediatric Endocrinology, Tor Vergata University, Room E-178, Via Montpellier 1, 00133 Rome, Italy.
E-mail: stefano.cianfarani@uniroma2.it

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born at term after an uneventful pregnancy. Birth weight was 2,400 g, and length 50 cm. No congenital malformations were recorded. She had normal breast development and menarche at the age of $14^{9}/_{12}$ years. She had a second and final period 5 months later. Her height was 148 cm (−2.3 SD), and midparental height was 157 cm (−0.8 SDS). Weight was 37.1 kg with 93% of ideal body weight. She had bilateral epicanthal folds. Intelligence was normal. Pubertal development was characterized by breast stage Tanner IV [Marshall and Tanner, 1969], but absence of both pubic and axillary hair.

Methods

Hormone analysis. Levels of testosterone, androstenedione, dehydroepindrosterone sulfate (DHEAS), estradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH), and thyroid hormones were assessed. Cortisol response to ACTH stimulation was tested. A level of 18 μg/dl or more at any time during the test was considered indicative of normal adrenal function.

Imaging studies. Ultrasonography was performed to evaluate uterus and ovary size and morphology. Skeletal development of left hand and wrist was assessed by X-rays to determine bone age according to Pyle et al. [1971].

Androgen receptor gene mutation analysis. As androgen receptor mutations may account for the absence of androgen-dependent characters such as axillary and pubic hair, we tested the patient for these mutations. This was performed as previously described by Lubahn et al. [1989]. Briefly, genomic DNA was isolated from peripheral blood leukocytes using kits from Qiagen (Milan, Italy). Exons 2–8 of the androgen receptor (AR) gene and flanking intron sequences were amplified using the Taq PCR Master Mix Kit (Qiagen). Sequencing of the PCR products was performed using the ABI Prism Dye terminator sequencing kit and the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Milan, Italy). Sequencing was repeated twice on new PCR products with antisense primers.

Cytogenetic analysis. Chromosome analysis was performed using the GTG banding technique on cultured peripheral lymphocytes from the proposita and her parents. Appropriate informed consent was obtained.

Array-CGH analysis. Sample DNA was isolated from blood with the QIAamp DNA Blood Midi Kit (Qiagen) and normal female DNA from Promega (Madison, WI) was used as a sex-matched control. DNA concentration was measured using a NanoDrop Spectrophotometer (NanoDrop Products, Thermo Scientific, Wilmington, DE), and genomic microarray analysis was performed using the Human Genome 4 × 44K whole-genome oligonucleotide arrays (75 kb resolution) (Agilent Technologies, Inc., Santa Clara, CA). DNA digestion, labeling, and hybridization were performed according to the manufacturer's instructions. The array was scanned using an Agilent Microarray Scanner with the Agilent Scanner Control software (Agilent Technologies, Inc.). Data were analyzed using Feature Extraction software v9.1 and CGH Analytics software v3.4 (Agilent Technologies, Inc.).

FISH analysis. To confirm the array-CGH result and to localize the breakpoint on chromosome 15q26, fluorescence in situ hybridization (FISH) analyses were performed using standard FISH procedure. BAC clones mapping to the regions of interest were selected using the UCSC genome browser (http://genome.ucsc.edu). BAC clones were grown in appropriate medium and DNA isolated, then labeled with Spectrum Orange or Spectrum Green using the Vysis Nick Translation Kit (Abbott Molecular, Inc., Des Plaines, IL). After hybridization, 4′,6-diamidino-2-phenylindole (DAPI) was used as a counterstain. FISH specimens were observed using an epifluorescence microscope Olympus BX61 and CytoVysion image analyzer system (Applied Imaging, San Jose, CA).

RESULTS

Plasma estradiol was initially found in the normal range. However, a second evaluation demonstrated subnormal concentrations (Table I). Both FSH and LH were abnormally elevated, thus suggesting a condition of hypergonadotropic hypogonadism (Table I). Ultrasonography of pelvis showed a pubertal uterus (anterior–posterior diameter 18 mm, cervical–fundus diameter 54 mm, latero-lateral diameter 31 mm) but with size reduced for the patient's age. The endometrial thickness was 3 mm. Ovarian size was reduced: left ovary volume was 1.6 ml, right ovary volume 1.4 ml, with no dominant follicles. Bone age was $13^{6}/_{12}$ years at the chronological age of $15^{11}/_{12}$.

TABLE I. Endocrine Evaluation of the Patient With Premature Ovarian Failure (POF)

Hormone	Patient	Normal values (adjusted for age and pubertal stage)
DHEAS	233 μg/dl	35–535
Andronstenedione	69 ng/dl	82 ± 47
Estradiol	44.0 and 10.04 pg/ml	21–85
FSH	72.23 mUI/ml	7.2 ± 1.4
LH	26.50 mUI/ml	7.9 ± 2.6
Total testosterone	37 ng/dl	15–40
Free testosterone	1.0 pg/ml	<2
Basal cortisol	21 mcg/dl	5–25
Cortisol after ACTH stimulation	38.7 μg/dl	>18
Basal 17α-OH-progesterone	0.9 ng/ml	1.6–2.3
17α-OH-progesterone after ACTH stimulation	3.0 ng/ml	<6.0

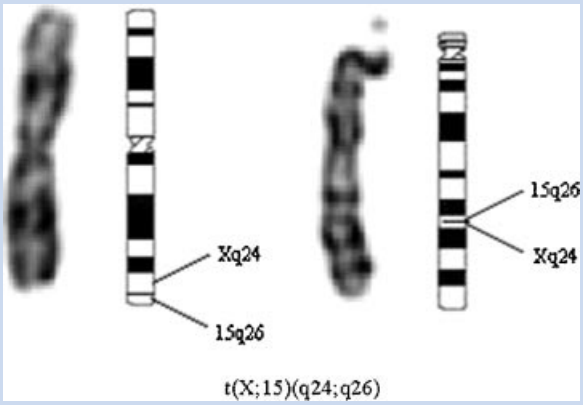

FIG. 1. Translocation of X;15 (q24;q26) by G banding (~400-band level).

No mutation in AR gene which is located on Xq11-12 locus was found. Chromosome analysis disclosed a translocation between chromosomes Xq and 15q, breakpoints were refined by molecular cytogenetic analysis using array-CGH and FISH (Fig. 1). The parents had normal chromosomes. Considering the proposita's phenotype and the presence of a de novo reciprocal translocation involving the long arm of chromosome X, we decided to perform high-resolution whole genome array-CGH analysis. This showed the presence of an Xq24 segmental duplication (A_14_P121231 → A_14_P102165). The extent of the duplication was approximately 675 kb (119,170,929 bp → 119,846,719 bp) (human genome build 17; Fig. 2). The dual-color FISH analysis using the contiguous BAC clones RP11-423F7 (green) and RP11-52A16 (red), mapping to the Xq24 region, confirmed the presence of the Xq24 duplication, showing three specific fluorescent signals for each BAC clone, respectively, on the normal X chromosome, on the Xq derivative, and on the 15q derivative (Fig. 3a,b). No structural aberration of the region of interest was present in the parents. Additional FISH analysis using BAC clones mapping to the 15q26 region localized the breakpoint to 15q26.3, between RP11-659J11 (not translocated) and RP11-327K11 (translocated) (Fig. 4a,b). Molecular analysis redefined the translocation

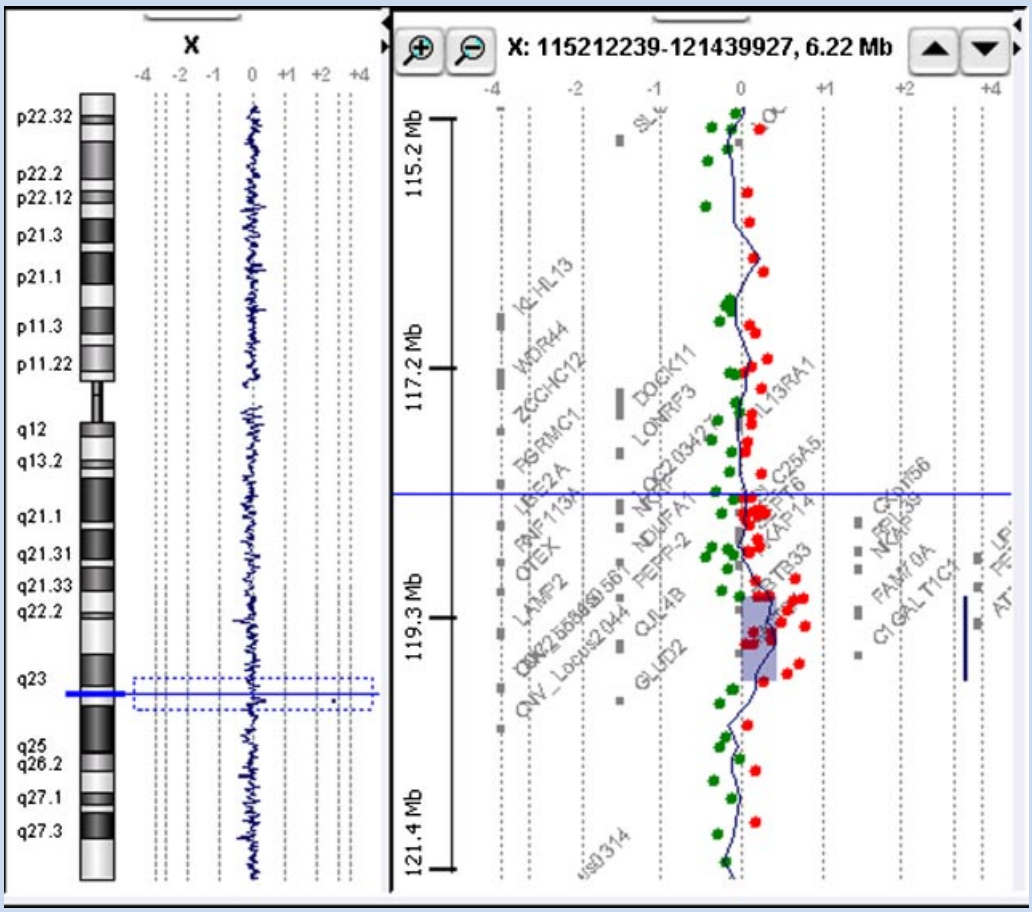

FIG. 2. Array-CGH analysis showing a 675-kb segmental duplication of the Xq24 region.

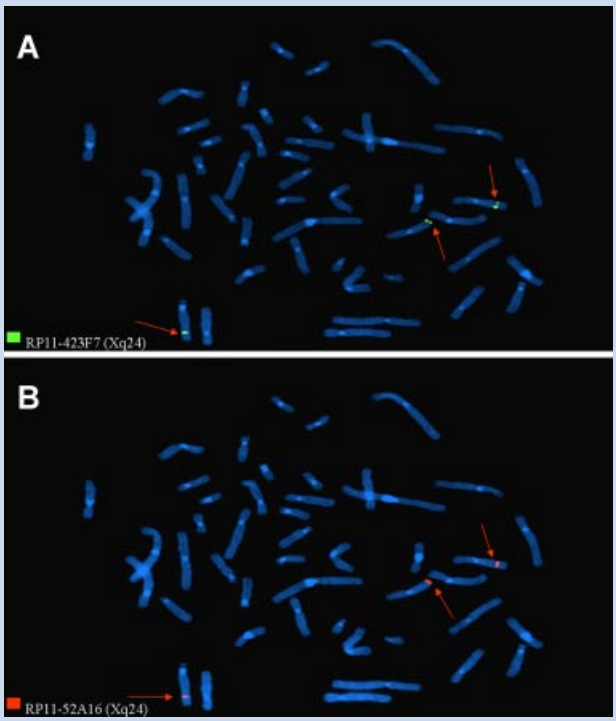

FIG. 3. FISH analysis using the contiguous BAC clones RP11-423F7 (green) (A) and RP11-52A16 (red) (B), mapping to Xq24, confirmed the presence of the duplication showing for each BAC clone three specific fluorescent signals: on the normal Xq chromosome, on the Xq chromosome derivative, and on the 15q chromosome derivative (arrows).

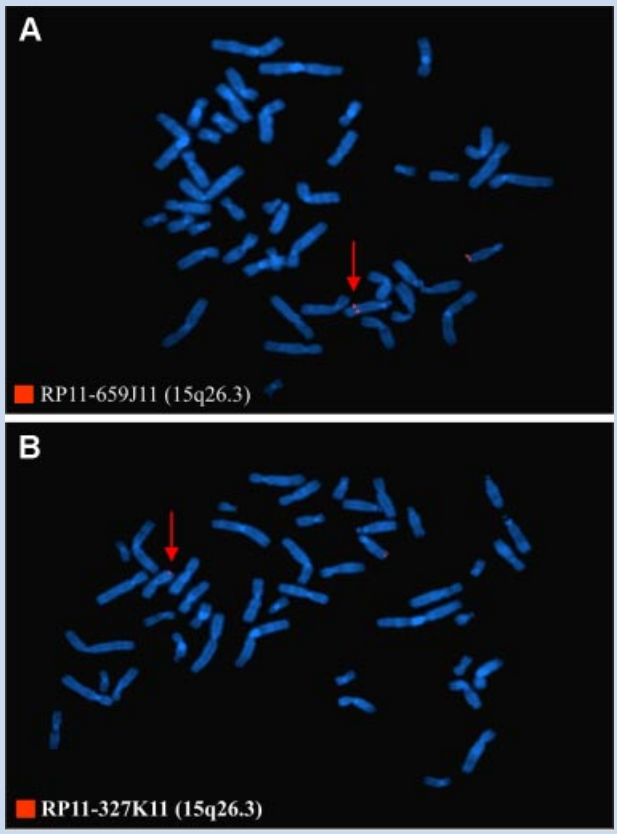

FIG. 4. FISH analysis localized the 15q breakpoint in 15q26.3, between BAC clones RP11-659J11 (not translocated) (A, arrowed) and RP11-327K11 (translocated) (B, arrowed).

breakpoints and the karyotype was: 46,X,t(X;15)(q24;q26.3).isht-(X;15)(q24;q26.3)(RP11-423F7+, RP11-52A16+, RP11-327K11+, RP11-659J11−; RP11-659J11+, RP11-327K11−, RP11-52A16+, RP11-423F7+) de novo.

DISCUSSION

Women whose menses cease prior to age 40 years and have menopausal FSH levels are considered to have POF. POF may be a manifestation of different underlying pathogenetic and causal processes. Many different genetic mechanisms are known, including X and autosomal chromosomal abnormalities, mutations of autosomal or X-linked genes, and polygenic/multifactorial determinants. In a number of POF cases balanced X chromosome rearrangements have been found, including inversions and X;autosome translocations [Therman et al., 1990].

Our patient with POF has a de novo unbalanced translocation X;15(q24;q26.3). To our knowledge, this is the first report of a young woman with an X;autosome translocation who presented with secondary amenorrhea and absence of pubic and axillary hair. In contrast to healthy children, in whom adrenarche is followed by gonadarche, dissociation between adrenarche and gonadarche has been reported in several endocrine disorders affecting either gonads or adrenals [Albright et al., 1942; Lee et al., 1975; Young et al., 1997; Ibanez et al., 2000]. This led to the assumption that the processes are independent of each other. The most obvious clinical consequence of adrenarche in girls is the growth of sexual hair, which is caused by conversion of adrenal androgens to (dihydro-)testosterone in the periphery and in the ovaries [Labrie et al., 2001].

Girls with Turner syndrome provided the first human model in which adrenal puberty occurs without gonadal puberty [Teller et al., 1986]. It was reported that primary gonadal failure in the Turner syndrome is associated with an earlier onset of adrenarche, rise of serum dehydroepiandrosterone sulfate (DHEAS), but delayed pubarche. These data suggest that normal timing of adrenarche depends on gonadal function and that normal pubarche is the clinical manifestation of the ovarian conversion of DHEAS to active androgens [Martin et al., 2004]. In contrast, our patient, though showing spontaneous pubertal development, showed pre-adrenarche levels of DHEAS without pubarche. Adrenal function was normal. The CYP-11A-P450SCC gene, that, if mutated is responsible for congenital adrenal insufficiency, is not included in the present translocation (it is located in the 15q23-q24 region).

The absence of pubic and axillary hair could suggest resistance to androgens. Therefore, considering the normal androgen hormone assessment, we ruled out a possible incomplete peripheral androgen

resistance due to AR gene mutation. The AR gene mutation analysis was normal.

Recently, the progesterone receptor membrane component-1 (PGRMC1) gene, located in the Xq22-q24 region, has been implicated in POF [Mansouri et al., 2008]. Mansouri et al. [2008] described two women, mother and daughter, with POF due to an X;autosome translocation involving Xq24 region. In both patients PGRMC1 mRNA expression and protein levels were downregulated, probably through a positional effect. PGRMC1 mediates the anti-apoptotic action of progesterone in ovarian cells, alterations in PGRMC1 function may hence result in premature ovarian follicle loss and, ultimately, in ovarian insufficiency. PGRMC1 acts also as a positive regulator of several cytochrome P450 (CYP)-catalyzed reactions [Mansouri et al., 2008]. CYPs are critical for intracellular sterol metabolism, including the biosynthesis of steroid hormones in adrenal glands. Mansouri et al. [2008] showed an abolished interaction between PGRMC1 and P450 enzyme CYP7A1 concluding that a PGRMC1 abnormal function may have an effect on several sterol metabolites. However, the patients described by Mansouri et al. did not show any clinical/endocrine features related to alterations of adrenal glands. In our patient, despite the absence of axillary and pubic hair, no alteration in adrenal steroid hormone levels was found. She was started on ethinylestradiol + gestodene therapy, and, after 4 months of therapy, she showed the appearance of both pubic (pubarche: Tanner stage II) and axillary hair. It seems reasonable to correlate the occurrence of pubarche with the administration of progestagens. Progestagens have androgen-like activity, although they also increase sex hormone binding globulin production leading to a reduction of circulating free testosterone [Fuhrmann et al., 1995]. It is tempting to speculate that PGRMC1 abnormal function could have caused an altered adrenal steroid metabolism leading to the blockade of pubic and axillary hair development which was partially reversed by gestodene treatment.

Our case demonstrates that distal translocation involving the POF1 locus is able to cause POF in adolescence and, so far, this is the first report of POF associated with complete absence of androgenization.

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