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## Cytogenetic abnormalities in Tunisian women with premature ovarian failure

*Anomalies chromosomiques et insuffisance ovarienne prématurée en Tunisie*

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## ABSTRACT

To identify the distribution of chromosome abnormalities among Tunisian women with premature ovarian failure (POF) referred to the department of Cytogenetic at the Pasteur Institute of Tunis (Tunisia), standard cytogenetic analysis was carried out in a total of 100 women younger than 40 affected with premature ovarian failure. We identified 18 chromosomal abnormalities, including seven X-numerical anomalies in mosaic and non-mosaic state (45,X; 47,XXX), four sex reversal, three X-structural abnormalities (terminal deletion and isochromosomes), one autosomal translocation and one supernumerary marker. The overall prevalence of chromosomal abnormalities was 18% in our cohort. X chromosome aneuploidy was the most frequent aberration. This finding confirms the essential role of X chromosome in ovarian function and underlies the importance of cytogenetic investigations in the routine management of POF.

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## R É S U M É

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L'insuffisance ovarienne prématurée (IOP) est une affection peu fréquente touchant 1 % des femmes âgées de moins de 40 ans. Elle est définie comme la présence d'une aménorrhée primaire ou l'apparition d'une aménorrhée secondaire avant l'âge de 40 ans et associe une hypooestrogénie et une élévation des gonadotrophines. Nous nous sommes proposé d'identifier les causes génétiques chez 100 femmes atteintes d'IOP adressées au laboratoire de cytogénétique de l'institut Pasteur de Tunis. Pour chaque patiente, un caryotype sanguin en bandes RHG a été réalisé. Nous avons décelé 18 % d'anomalies

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chromosomiques touchant essentiellement le chromosome X (anomalies de nombre et/ou de structure), quatre cas de réversion de sexe et une translocation autosomique. Ces chiffres incitent à la considération de l'étude cytogénétique au cours du bilan de l'insuffisance ovarienne prématurée, encore non systématique dans notre environnement.

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## 1. Introduction

Premature ovarian failure (POF, OMIM 311360) is defined as the cessation of ovarian function before the age of 40, associated with elevated gonadotrophin serum levels [1]. The diagnostic is based on elevated FSH in a menopausal range (usually  $\geq 40$  IU/l) detected on at least twice within a few weeks (Conway 2000). POF occurs in one per 1000 women aged 30 and in one per 100 women by aged 40. This disorder may be characterized by primary amenorrhoea or secondary amenorrhoea for at least 4–6 months and is considered as a common pathology leading to infertility [2]. In fact, POF leads to 10% of ovulatory female sterility [1]. POF can be observed as a syndromic form associated with other features or as an isolated condition. The etiology of POF is highly heterogeneous, including genetic, metabolic, infectious, autoimmune, and iatrogenic (anticancer treatment) causes [3]. The most common genetic cause of POF is X chromosome abnormalities ranging from numerical defects, deletions, X-autosome translocations, and isochromosomes [3–5]. Chromosomal abnormalities have been recognized as a frequent cause of POF, with varying percentages in reported series [6–8]. However, in a large proportion of cases, no etiology is found and POF is classified as idiopathic [3].

The aim of this study was to investigate the frequency and type of chromosome abnormalities in Tunisian POF patients in order to assess the efficacy of cytogenetic screening.

## 2. Patients and methods

### 2.1. Patients

Between January 2002 and December 2012, 100 POF patients were referred by clinicians for cytogenetic analysis to the Cytogenetic Department at the Pasteur Institute of Tunis.

Inclusion criteria were primary amenorrhoea or secondary amenorrhoea for more than 4 months prior to the age of 40, with FSH serum levels higher than 40 IU/L. All of the patients referred to our department underwent a complete clinical assessment, including complete medical and gynaecological history, in order to exclude any other related pathology. Women with clinical signs of Turner's syndrome were excluded as well as women with personal history of autoimmune disease or clinical antecedents of pelvic surgery. Written informed consent was obtained from all participants.

### 2.2. Methods

#### 2.2.1. Conventional cytogenetic analysis

Metaphase chromosome spreads were obtained from phytohaemagglutinin-stimulated peripheral blood

lymphocytes. Karyotype analysis was performed on RHG-banded metaphase chromosomes using a standard protocol that generated 500–550 band resolutions. A minimum of 20 metaphases per patient were analyzed. If any cell among the 20 showed a non-model cell (45,X or 47,XXX), an additional 30 cells were counted. Chromosome polymorphisms, for example pericentric inversion of chromosome 9 and centromeric heterochromatin variants, were recorded, but classified as normal. Chromosomal abnormalities have been reported in accordance with the current international standard nomenclature (ISCN 2009).

#### 2.3. Fluorescent *in situ* hybridization (FISH) analysis

FISH study was performed using alpha satellite probes of chromosome X and Y (alpha satellite DXZ 1 probe/Green Q Biogen, chromosome Y alpha satellite DYZ 3 probe/Red Q Biogen) and using LSI SRY, p11.3 Spectrum Orange, Abbott-Vysis. The application of the probes was done according to the manufacturer's instructions. A range of 100 nuclei and metaphases were taken into account.

## 3. Results

One hundred POF patients were included in this study. The average age was  $26.95 \pm 6.4$  years (16–40) at the time of cytogenetic exploration. Most of our patients ( $n = 60$ ; 60%) presented with secondary amenorrhoea (SA), while the others presented with primary amenorrhoea (PA).

Table 1 shows the characteristics of each group. These patients do not present any specific somatic anomalies, except for one woman who displayed a blepharo-phimosis epicanthus syndrome.

We detected 18 chromosomal abnormalities (18%) using karyotype analysis. Among these patients, one case showed an autosomal abnormality, another woman had a supernumerary marker at a mosaic state and 16 cases had gonosomal abnormalities (88.8%). The rest of patients (82) had normal karyotype.

The frequency of karyotypic abnormalities in patients with PA (13/40, 32.5%) was higher than the frequency of patients with SA (5/60, 8.3%).

The distribution and details of chromosomal abnormalities are summarized in Table 2.

The most common abnormality was numerical X chromosome abnormalities, which were found in seven cases (38.8%). Moreover, we detected different kinds of structural X chromosome abnormalities: one X(q) deletion, one isochromosome [i(Xq)] and one mosaic isodicentric that was confirmed by FISH. Furthermore, in this latter case, FISH allowed the detection of a monosomic X cell line in a proportion of 12% of observed cells.

**Table 1**  
Characteristics of 100 of Tunisian women with POF.

Characteristic	Primary amenorrhoea (n = 40)	Secondary amenorrhoea (n = 60)
Age at diagnosis (years)	24.2 ± 6.04	28.8 ± 6.06
Age at menarche (years)	–	13.4 ± 1.62
Age of amenorrhoea (years)	–	23.8 ± 6.6
BMI (kg/m <sup>2</sup> )	21.78 ± 3.02	24.48 ± 4.2
FSH (UI/L)	41.8 ± 40.12	66.85 ± 34.6
LH (mUI/mL)	12.16 ± 10.9	35.32 ± 30.14
E2 (pg/ml)	47.7 ± 44.7	43.8 ± 33.9

FSH: follicle-stimulating hormone, LH: luteinizing hormone.

In six cases, a Y chromosome was found: four non-mosaic 46,XY, one 45,X/46,XY and the latter 45,X/46,X, idic(Yp). The main clinical feature seen in 46,XY women was a moderately tall stature, whereas the patient with a mosaic 45,X/46,XY has a growth delay (152 cm). The patient with the 45,X/46,X, idic(Yp) karyotype presented a PA, a pubertal delay. FISH analysis with Y chromosome alpha satellite DYZ 3 probe confirmed the dicentric nature of the abnormal Y chromosome. The analysis of 100 metaphases showed a fluorescent signal in 20% of metaphase spreads corresponding to the idic(Y) cell line. Hybridization with LSI SRY probe at Yp11 showed a double fluorescent signal at the abnormal Y chromosome.

Finally, a reciprocal translocation between the long arm of chromosome 12 and 19 was revealed at a non-mosaic state in phenotypically normal woman having a SA.

#### 4. Discussion

POF is a heterogeneous disorder. In most cases, POF is idiopathic and its underlying mechanisms are largely unknown [9]. However, diverse etiologies have been associated with POF, including genetics factors. An

**Table 2**  
Detailed list of chromosomal aberrations among the study population.

	No. of cases	Type of amenorrhoea
X-numerical anomalies		
45,X	1	PA
45,X[1]/46,XX[29]	1	PA
45,X[1]/46,XX[18]	1	SA
45,X[23]/47,XXX[27]	1	SA
47,XXX	1	PA
46,XX[48]/47,XXX[2]	1	SA
46,XX[23]/47,XXX[2]	1	PA
X-structural anomalies		
46,X,del(Xq)	1	PA
46,X,i(X)(q10)	1	PA
46,X,i(X)(q11)[19]/47,X,i(X)(q11),i(X)(q11)[1]	1	PA
46, XY lines		
46,XY	4	PA
45,X[15]/46,XY[35]	1	PA
45,X[80]/46, idic(Yp)[20]	1	PA
Autosomal anomalies		
46,XX,t(12;19)(q13;q13)	1	SA
Supernumerary Marker		
46,XX[10]/47,XX,+ mar [2]	1	SA

PA: Primary amenorrhoea, SA: secondary amenorrhoea.

association between POF and abnormalities of the X chromosome has been extensively reported in the literature [3].

Cytogenetic analysis is an important tool for the detection of cytogenetic abnormalities that lead to premature ovarian failure. In this study, we identified 18 chromosomal abnormalities out of 100 cases of POF referred to our department. Chromosomal abnormalities have been estimated to occur in 10% to 25.3% of women with POF [6,10–13]. The overall frequency of chromosomal abnormalities in our study is compared to reported frequencies from different previous studies in Table 3.

The prevalence is significantly higher in patients with PA compared with patients with SA, as reported in many studies in the literature [6,12,13].

Our data show a high prevalence of X chromosome abnormalities (16 cases out of 18 found, equal to 88.8%), highlighting, as described in the literature, the importance of X chromosome in ovarian function and POF cases etiologies [3,4,10]. These abnormalities ranged from the complete absence or trisomy of the X chromosome to deletion of genetic material of the long arm of the X (46,X,del(X)(q21)) or an isochromosome of the long arm: iso(Xq). Several cytogenetic studies have demonstrated the implication of the Xq in defects of ovulation more than Xp. Two critical regions (CR) for POF: CR I; Xq13–Xq21 and CR II Xq23–Xq27 have been reported in the cytogenetic and molecular characterization of X rearrangements [7,14–16].

Mosaicism with a 45,X line was common overall and was the most frequently detected anomaly in our study. It was described in five out of 18 anomalies found in our 100 patients. In addition to arising from mitotic non-disjunction, a monosomy X line can arise secondarily if an X-structural abnormality exists, especially a dicentric chromosome or an isochromosome as found in one of our patients that was detected by FISH analysis.

Non-mosaic 47,XXX was found in one POF case, an association reported previously [17], but which still remains of uncertain significance. In our series, the prevalence of 47,XXX was 3% including two mosaic cases (with 46,XX line) in accordance with the prevalence found by Goswami et al. (3.8%) [18]. The presence of three X chromosomes could lead to meiotic disturbance resulting in plausibly ovarian failure and/or in an overexpression of genes escaping X-inactivation.

The presence of Y-bearing cells was unexpectedly found in 6 “POF” patients. All cases had PA and female external genitalia. The 46,XY female is rare but not exceptional cause of primary amenorrhoea. In addition to complete (or partial) XY gonadal digenesis related to a SRY gene

**Table 3**  
Summary of frequency of chromosomal abnormalities in different population studies of POF.

Number of patients	Populations	Chromosomal abnormalities (%)	References
100	Tunisian	18	Our study
179	Iranian	10.05	[1]
531	Chinese	12.1	[6]
269	Italian	10.0	[11]
75	Turkish	25.3	[13]

mutation, 46,XY sex reversal may occur in association with renal abnormalities, due to *WT1* gene mutation [19], adrenal insufficiency (associated with *SF1* gene mutation) [20], or campomelic dysplasia (alteration of the *SOX9* gene) [21].

Moreover, 45,X line were found in two 46,XY women at mosaic state. The phenotypic range of variability of 45,X/46,XY mosaicism extends from Turner syndrome to mixed gonadal dysgenesis to normal males [22]. This variability is primarily dependent on the dominant cell line present in the developing gonad. Structurally abnormal Y chromosomes are often seen in mosaicism with 45,X cell line [22]. The associated phenotype displays wide variability. In our study, one patient was referred for PA and a short stature had a 45,X/46,X,idic(Yp) at the karyotype. Kelly et al. reported two patients with a similar karyotype: one patient presented with TS, the other with mixed gonadal dysgenesis. Despite the karyotypic similarity of these two patients, the differences in the observed phenotype were explained by molecular differences.

Autosomal translocations are uncommon in women with POF. To the best of our knowledge, very few autosomal translocations, Robertsonian or reciprocal, have been reported in POF [6], [11–13]. We detected a single reciprocal translocation: in our case, breakpoints were located in 12q13 and 19q13. Molecular and genome-wide association studies (GWAS) have demonstrated that some candidate genes (*BRKS1* and *MCM*), Copy Number Variants (CNVs) and Single Nucleotides Polymorphisms (SNPs) located in 19q13 were involved in steroidogenesis, estradiol signalling and associated with the natural age at menopause, suggesting that this autosomal abnormality could explain the POF in our patient [23]. Therefore, further defining of breakpoint by array *Comparative Genomic Hybridization* (CGH array) is of importance in exploring the genetic etiology of POF associated with this autosomal translocation [24].

## 5. Conclusion

In conclusion, the relationship between chromosomal abnormalities and POF is clearly demonstrated in the present study, in which a genetic cause was found in 18% of Tunisian women with POF. Our results confirm the importance of X chromosome in POF etiology, highlighting the importance of routine assessment of chromosomal anomalies. This analysis helps to provide valuable clinical information for reproductive management and genetic counseling. Cytogenetic investigations should become a part of the routine management of POF regardless of patient age, and even when there are no clinical features suggestive of chromosomal abnormalities.

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