



# Interphase chromosome profiling (ICP) identifies Y-chromosome origin of a ring(X) chromosome called by G-band pattern in a patient with Turner syndrome

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## Abstract

Turner syndrome (TS) is a chromosomal disorder affecting one in about 2000 female births. Although the typical chromosome abnormality is the absence of the second X-chromosome in-whole or in-part, abnormalities of Y-chromosome ranging from the presence of normal XY cells in mosaicism with XO cells to structurally abnormal Y have been reported in these patients. Y-sequences have been reported in about 5% of TS patients by PCR analysis using Y-specific genes. TS patients with Y-chromosome are at an increased risk for developing gonadoblastoma (GB) and therefore require gonadectomy. This risk for malignancy necessitates the unequivocal identification of markers or ring chromosomes in these patients for proper management. Here we present a case with TS in which the G-band ring(X) was subsequently identified as a ring Y-chromosome by ICP assay.

The patient is a 34 year old G0P0 referred for infertility evaluation. She reported an absence of natural menarche, but she was subsequently able to have menstrual cycles with oral contraceptive initiation. However, when she stopped the treatment, her amenorrhea returned. Past medical history is significant for excision of a 3kg ovarian tumor as a teenager. Physical features included a height of 4'8.69", webbed neck and cubitus valgus. Chromosome analysis of G-banded metaphases from PHA stimulated peripheral blood showed mosaicism for 45,X[11]/46,X,r(X) [9] karyotype. ICP assay was performed with X-chromosome probes to precisely map the chromosome breaks that led to the development of the r(X). All interphase nuclei (200) evaluated had one copy number for all probes, and evaluation of metaphases did not show signals on the r(X)(Fig.2). ICP was repeated with Y-chromosome probes; this assay identified the ring as derived from a Y-chromosome with breaks in short arm (proximal to Yp11.2) and in long arm (distal to Yq11.23). This was further confirmed by standard FISH with SRY and Y-centromere probes which showed loss of SRY consistent with the ICP results.

ICP is a new molecular cytogenetic technology. In this method FISH probes cover the entire length of each chromosome at an approximately equidistant points and this allows for a resolution equal to at least 600 G-bands on metaphase. Each probe is labeled with a different color to enable distinction from the adjacent probe. Signal patterns for different probes on each homologue can be traced to a chromatin thread in an interphase nucleus (Cytogenet Genome Res 2014;142:226, Abstract 22). Unlike G-bands, the position of these probes is molecularly precise, therefore ICP provides precise molecular information on the genomic position of structural alterations.

Early confirmation of the presence of a Y-chromosome derived genome is critical to the care of patients with TS because about 35.3% of these patients can develop GB. GB is a benign tumor with excellent prognosis if detected early. However, it has the potential to progress to dysgerminoma with metastatic potential. Prophylactic gonadectomy is therefore required. The peri-centromeric (Yp11-Yq11) GonadoBlastoma on the Y-chromosome (GBY) region with genes such as TSPY1 is implicated in the genesis of this tumor. Standard karyotype of the patient in this study had a ring sex chromosome which in size and by G-band pattern appeared to be r(X). ICP study traced the origin of this ring to Y chromosome with deletion in the distal short and long arms followed by the fusion of the centric fragment. Therefore, this abnormal Y-chromosome retained the GBY region and this may have contributed to the ovarian tumor excised during adolescence. Earlier methods of detecting the presence of Y-derived genome in TS patients used PCR methods with the probes for centromere and for genes in Yp11-Yq11 region; this method is not accessible by typical cytogenetics laboratories. ICP is an alternative to the PCR method as it defines the breaks in the Y-chromosome at the molecular level and it is accessible in all cytogenetics laboratories.

## Introduction

The typical chromosome abnormality in Turner syndrome (TS) is the absence of the second X-chromosome in whole or in part, particularly the short arm. Abnormalities of the Y-chromosome ranging from the presence of normal XY cells in mosaicism with XO cells to structurally abnormal Y missing the sex determining region have been reported. In the absence of cytogenetically detectable Y-chromosome, Y-specific gene sequences have been reported in about 5% of TS patients by PCR analysis. TS patients with a Y-chromosome are at an increased risk for development of gonadoblastoma (GB) and therefore require gonadectomy. This risk for malignancy necessitates the unequivocal identification of markers or ring chromosomes in these patients for proper management. Here we present an assay and an inexpensive method, called interphase chromosome profiling (ICP), to trace the origin of a G-band ring X-chromosome in a patient with TS.

## Case

The patient is a 34 Y G0P0 referred for the evaluation of infertility. She reported an absence of natural menarche, but she was subsequently able to have menstrual cycles with oral contraceptive initiation. However, when she stopped the treatment, her amenorrhea returned. Past medical history is significant for excision of a 3kg ovarian tumor as a teenager. Physical features included a height of 4'8.69", webbed neck and cubitus valgus.

## The ICP method design and probes

This method employs chromosome band specific DNA probes labeled with different fluorochromes, and these are placed at an approximately equal distance covering the landmark G-bands. Probes for centromeres and telomeres are labeled with single fluorochrome, whereas the probes for the interstitial bands are labeled with either single color or with dual colors. After hybridization and washing, signals from the probes can be traced to the location of the chromatin thread or the chromonema of a chromosome, and therefore constructing an interphase karyotype is feasible. A numerical variation produces more (trisomy) or less (monosomy) signals for the entire chromosome, a heterozygous deletion results in loss of one or more signals in one chromonema, a translocation results in the dislocation of a group of signals from the main chromonema in the interphase nucleus. This interphase karyotype gives a resolution at a 600 G-band level. Therefore, structural alterations can be precisely defined, and the origin of marker chromosomes can be identified.

## Result

Chromosome analysis of G-banded metaphases from PHA stimulated peripheral blood showed mosaicism for 45,X[11]/46,X,r(X) [9] karyotype (Fig.1). The ICP assay was performed with X-chromosome probes including a probe for X-centromere to precisely map the chromosome breaks that led to the development of the r(X). All interphase nuclei (200) evaluated had one copy number for all probes, and evaluation of metaphases did not show signals on the r(X)(Fig.2). ICP was repeated with Y-chromosome probes; this assay identified the ring as derived from a Y-chromosome with breaks in the distal short (Yp11.31) and long arm (Yq12)(Fig.3). This was further confirmed by standard FISH with SRY and Y-centromere probes which showed loss of SRY (Fig. 4) consistent with the ICP results.

Fig.1: G-band karyotype showing a ring chromosome



Fig.2. ICP for X chromosome: one normal X signals, absence of X signals on the ring chromosome

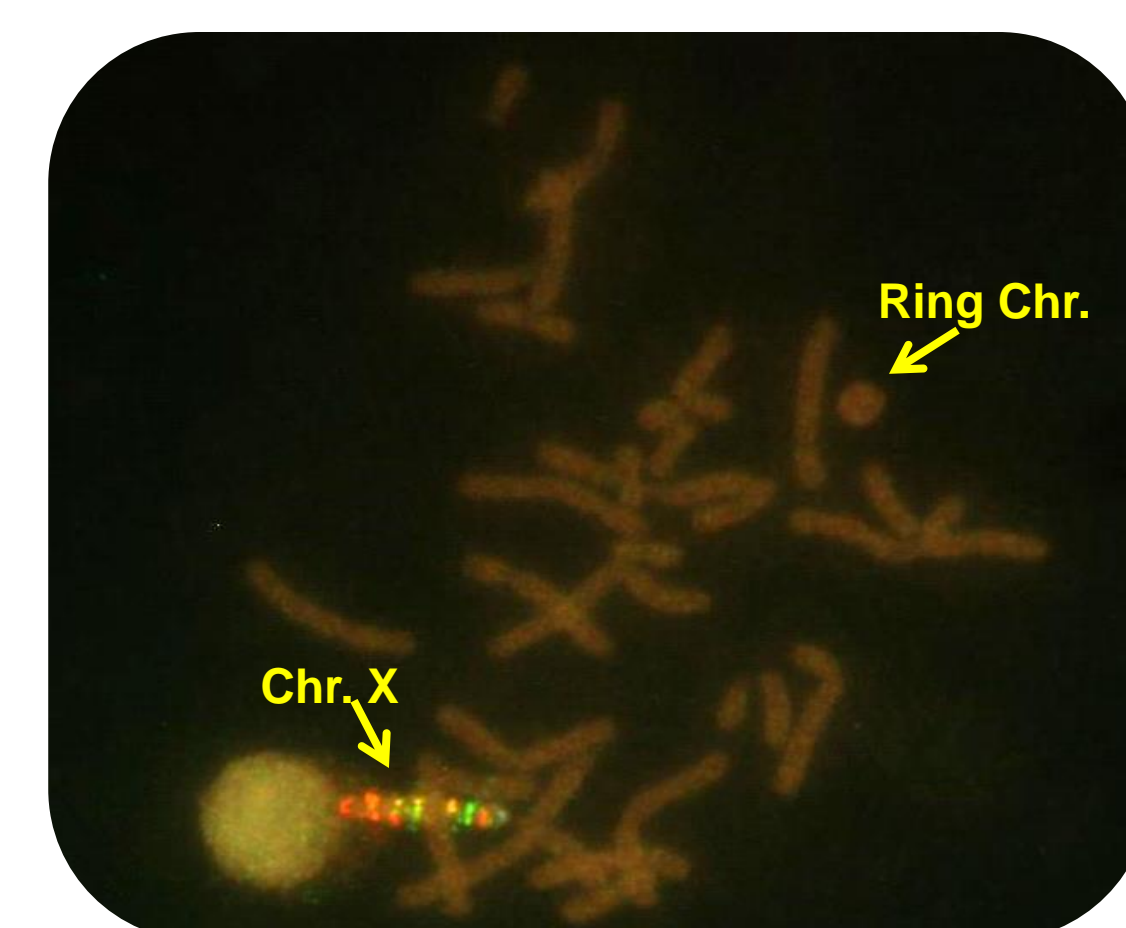


Fig.3. ICP for Y chromosome: presence of Y signals (red and cy3) in the ring chromosome. Missing the Yp11.2 (aqua signal)-SRY region

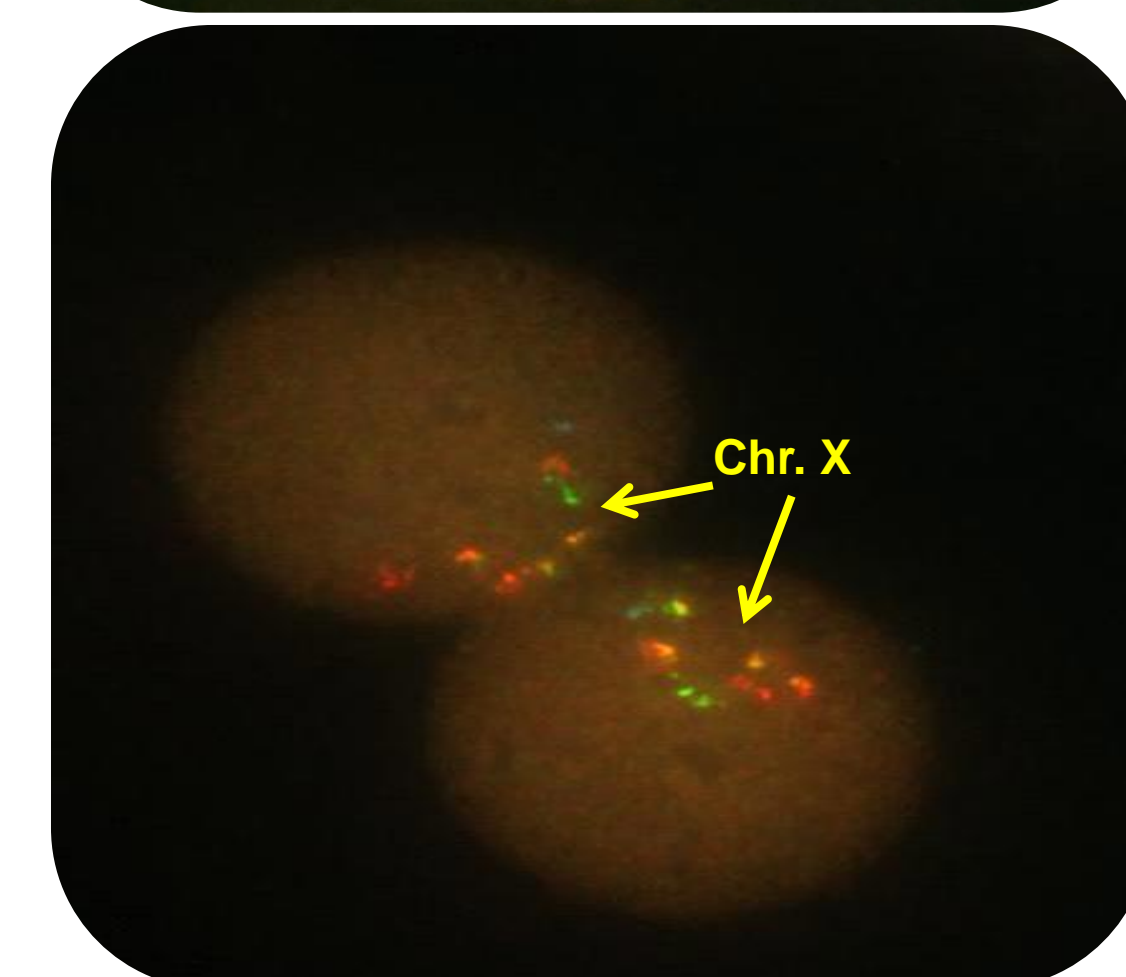
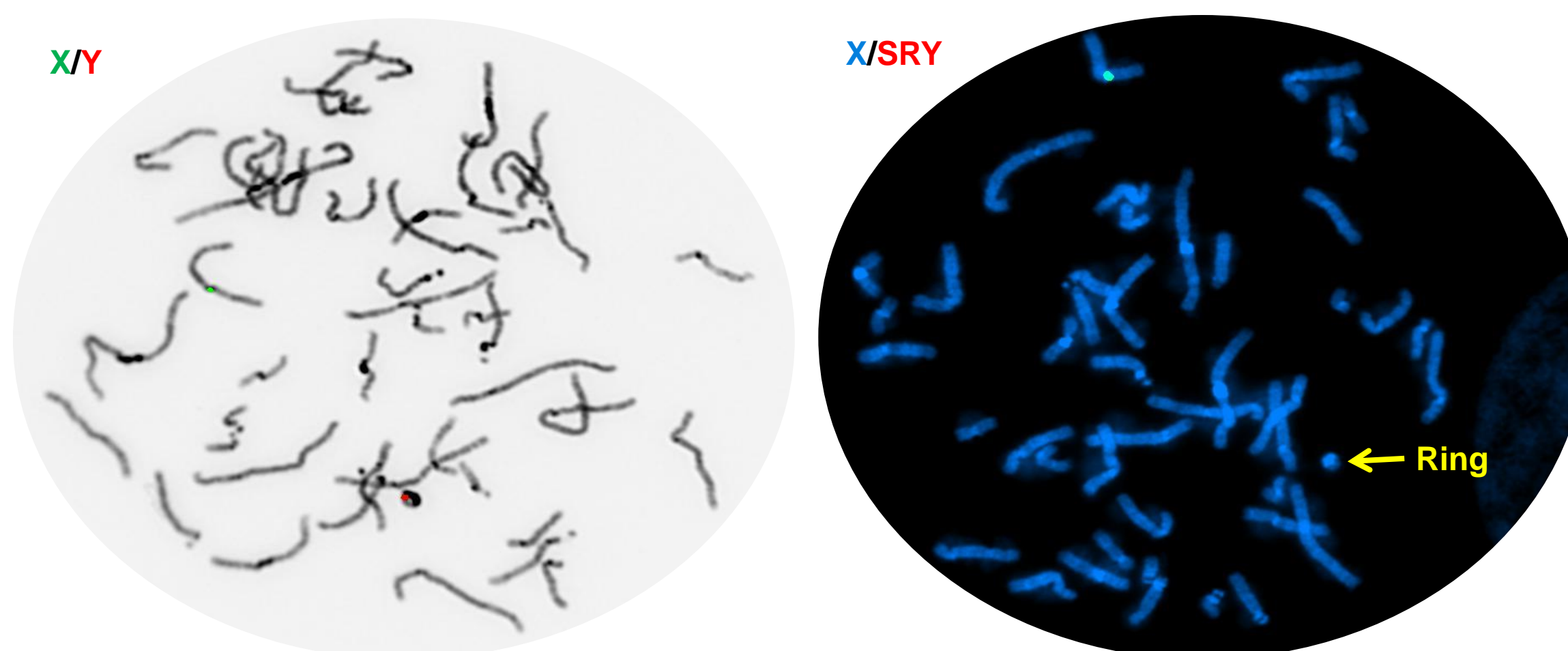


Fig.4. Standard FISH confirmed the presence of X and Y centromere and loss of SRY region



## Discussion and Significance

Early confirmation of the presence of a Y-chromosome derived genome is critical to the care of patients with TS because about 35% of these patients can develop GB. GB is a benign tumor with excellent prognosis if detected early. However, it has the potential to progress to dysgerminoma with metastatic potential. Prophylactic gonadectomy is therefore required.

The peri-centromeric (Yp11-Yq11) GonadoBlastoma on the Y-chromosome (GBY) region with genes such as TSPY1 is implicated in the genesis of this tumor. Standard karyotype of the patient in this study had a ring sex chromosome which in size and by G-band pattern appeared to be r(X). ICP study traced the origin of this ring to a Y chromosome with deletion in the distal short and long arms followed by the fusion of the centric fragment. Therefore, this abnormal Y-chromosome retained the GBY region and this may have contributed to the ovarian tumor excised during adolescence. Earlier methods of detecting the presence of a Y-derived genome in TS patients used PCR methods with the probes for centromere and for genes in Yp11-Yq11 region. However, this method is not accessible by typical cytogenetics laboratories. ICP is an alternative to the PCR method as it defines the breaks in the Y-chromosome at the molecular level and it is accessible in all cytogenetics laboratories.

## References

- Oliveira RM, Verreschi IT, Lipay MV, Eça LP, Guedes AD, Bianco B. Y chromosome in Turner syndrome: review of the literature. Sao Paulo Med J. 2009 Nov;127(6):373-8. Review.
- Interphase chromosome profiling (ICP): development and validation of a novel technology and its clinical applications. Babu VR, Dev VG, Koduru, P. et al Cytogenet Genome Res 2014; 142, 226, abstract 22.
- Interphase chromosome profiling in the workup of product of conception and hematological malignancies. Time to do away with classical karyotype. Babu VR, Dev VG, Koduru P. et al. ACMG meeting 2015, abstract 181, Salt Lake City, Utah