An Atypical Turner Syndrome Patient with Ring X Chromosome Mosaicism*

EDUARDO S. CANTÚ, Ph.D.,† DONNA F. JACOBS, B.S.,† and G. SHASHIDHAR PAI, M.D.‡

†Cytogenetics Section, Department of Pathology and Laboratory Medicine, and ‡Division of Genetics and Child Development, Department of Pediatrics, Medical University of South Carolina, Charleston, SC 29425

ABSTRACT

Small marker chromosomes (SMC) associated with severe Turner syndrome (TS) variants often represent reduced X chromosomes lacking the X inactivation center (XIC), perturbed dosage compensation, and unbalanced gene expression. A TS patient with mental retardation (MR), unusually short stature, facial and limb malformations, and karyotypic mosaicism involving SMCs is described. Cytogenetic and fluorescence in situ hybridization (FISH) studies of blood and lymphoblastoid cells showed that the SMC was X-chromosome derived, contained a functional centromere, and had ring formation. Karyotypes of 45/46,X,r(X) in blood cells and 45,X/46,-XX/46,X,r(X)/47,X,r(X), + r(X) in fibroblasts were found. Late-replication of the SMC was inconclusive, but the X inactivation specific transcript (XIST) locus within XIC was demonstrated by fluorescent in situ hybridization (FISH). Mechanisms are reviewed that can account for our patient's unusual TS phenotype.

Introduction

Turner syndrome (TS) is associated with complete or partial monosomy of the X chromosome and occurs with an incidence of about 1 in 5,000 human female live births. It is highly lethal in utero, and only 1.0% of all 45,X conceptuses are thought to survive. Subsequently, this sex chromosome imbalance is characterized by relatively benign physical stigmata which include short stature, broad chest with widely spaced nipples, characteristic facial features, gonadal dysgenesis, webbed neck, low posterior hairline, and high incidence of renal and cardiovascular anomalies.1

Although some studies have suggested a specific cognitive deficiency with visual perceptual deficits,2 the incidence of mental retardation (MR) does not appear to be higher in TS than in the population at large, except in a subgroup of patients who have mosaicism involving small marker chromosomes (SMC).3

* Send reprint requests to: Eduardo S. Cantú, Ph.D., Cytogenetics Section, Department of Pathology and Laboratory Medicine, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425.
Dyke et al\textsuperscript{3} have proposed that MR, and perhaps other features uncharacteristic of TS, may be due to the presence of inappropriately activated small r(X) chromosomes which lack the XIC. Other studies have associated SMC with MR\textsuperscript{4,5} with mosaicism\textsuperscript{6} and other severe phenotypes\textsuperscript{7} when they were r(X) chromosomes, but with risk for gonadoblastoma\textsuperscript{8} when they were Y chromosome derived. In light of the present understanding of SMC in TS individuals, a TS patient with MR and a diminutive body has been reviewed. Our findings from thorough clinical assessment, conventional cytogenetic analysis, and fluorescent \textit{in situ} hybridization (FISH) studies using X and Y chromosome specific DNA probes, are reported and their significance is evaluated in terms of how they may be related to MR and other severe characteristics found in TS.

Clinical Report

A 19 year old Caucasian female (figure 1) diagnosed at age 5 years with mosaic Turner syndrome on the basis of short stature, triangular face, large ears, webbed neck, and shield shaped chest with laterally displaced nipples. She was born at term weighing only 4 lb, 12 oz, being considerably smaller than her older sister whose birth weight was 7 lb, 9-\(\frac{1}{2}\) oz. Flexion contracture of several fingers had been noted at birth, and orthopedic evaluation undertaken because of delay in walking led to the diagnosis of hip dislocation in the second year of life. She had short fourth and fifth metacar-

\textbf{FIGURE 1.} Photograph of patient at age 19 years showing (a & b) typical triangular face and large ears, c) flexion contractures of the hand, and d) toe anomalies in both feet.
pals, and, unexpectedly, severe shortening, mal-
implantation, and lateral deviation of the toes on
both of her feet.

Other features not usually seen in Turner syn-
drome were present in this child. These included
extreme short stature which was non-responsive to
growth hormone treatment, strabismus, scoliosis,
mental retardation, and mitral regurgitation, pre-
sumed to be secondary to rheumatic heart disease.
Thromboembolic episodes secondary to mitral
valve prosthesis left her with mild left-sided hemi-
paresis. At 17 years of age, her most recent psycho-
logical evaluation showed her cognitive abilities to
be in the range of moderate mental retardation. She
has had intractable hypertension, possibly second-
ary to renal vascular stenosis associated with horse-
shoe shaped kidneys.

Methods and Materials

CYTOGENETIC STUDIES

Methanol/acetic acid fixed metaphase
cells from peripheral blood, lymphoblasto-
toid cell cultures, and skin fibroblast cul-
tures derived from our patient, were
studied by the conventional GTG-
binding technique.9 Peripheral blood
and lymphoblastoid cells were further
characterized by CBG-banding,10
Ag-NOR staining,11 and late-replication
studies.12 These techniques were
applied in order to characterize the
nature and extent of the karyotypic mosa-
icism found, and also to determine the
origin and the structural configuration of
the SMC.

FLUORESCENCE IN SITU
HYBRIDIZATION (FISH)

Cytological materials from blood and
lymphoblastoid cell cultures, prepared as
described for routine cytogenetic studies,
were evaluated by a modified FISH pro-
tocol previously reported.13 Three sex
chromosome derived non-isotopically
labelled deoxyribonucleic acid (DNA)
probes commercially available* were
used for the in situ hybridization experi-
ments. These included DNA probes with
sequence homology to locus DXZ1 (X
chromosome specific centromeric DNA),
to locus DYZ3 (Y chromosome specific
centromeric DNA), and to the X inactiva-
tion specific transcript gene (XIST).14
The X and Y alphoid (centromeric) DNA
probes are chromosome specific subsets
of human alpha-satellite repetitive DNA
family which have related sequence
homology.15,16,17 The method of non-
isotopic probe labelling of these probes
was by incorporation of derivatized nucleotides using nick translation.18

After the DNA probes are labelled, the
essential steps of the general FISH pro-
tocol include: RNase treatment of fixed
metaphase cells; denaturation of the
probe DNA and of previously prepared
metaphase chromosome DNA; in situ
hybridization of the non-isotopically
labelled probe DNA to chromosomal
DNA; removal of non-specifically bound
DNA by using specific washing condi-
tions; detection of hybridized probe
DNA by reacting with fluorescein
isothiocyanate (FITC) labelled avidin
(for biotin labelled DNA probes) or with
FITC labelled antibody (for digoxigenin
labelled DNA probes); and, if necessary,
amplification of the signal by further
reaction with fluorescein conjugated
antibodies. Assessment of localized fluo-
rescein signals after counterstaining with
propidium iodide was conducted by epi-
fluorescence microscopy using a Zeiss
photomicroscope II with a HBO 50 watt
DC mercury lamp and filters having max-
imums of 492 nm and 520 nm absorption
and emission, respectively.

Results

Cytogenetic GTG-banding studies of
peripheral blood, lymphoblastoid cells,
and cultured skin fibroblasts, showed the presence of mosaicism involving a SMC (smaller than a chromosome 20) in the form of a ring (figure 2a). The CBG-banding suggested the presence of a centromere in this SMC (figure 2b); Ag-NOR staining showed no evidence of a nucleolus organizer region, and late replication studies were inconclusive owing to the small size of the SMC. Furthermore, cytogenetic studies of the skin fibroblast culture detected a single cell with a normal 46,XX karyotype and an additional cell line with a second ring chromosome presumed to be X chromosome derived.

Fluorescent in situ hybridization studies of blood and lymphoblastoid cells identified the ring chromosome to be X chromosome derived since signals were found over the ring only when X chromosome specific DNA probes (DXZ1 and XIST) were used with no signals found with Y chromosome specific DNA probes.

Mosaicism was also confirmed when the X chromosome specific probes were used. Two major cell categories were evident, one with single signals, representing cells with only one X chromosome and no r(X), and one with double signals representing cells with both an X and a r(X) chromosome (figure 3a). The FISH studies using the XIST DNA probe yielded positive signals not only over the expected Xq13.3 locus of the normal X chromosome, but also over the r(X) chromosome (figure 3b).

Information collected from both the cytogenetic and FISH studies showed 45,X/46,X,r(X) mosaicism in peripheral lymphocytes and transformed lymphoblasts with presence of the XIST locus on the r(X). In fibroblasts, cytogenetic studies showed 45,X/46,XX/46,X,r(X)/47,X, r(X),+r(X). The FISH studies were not conducted on fibroblast cells.

Discussion

Since there is quantitative disparity between the sexes with respect to the gene loci on human X and Y chromo-

---

**Figure 2.** Photomicrographs of partial metaphases showing (a) ring formation of the small marker using GTG-banding, and (b) positive C bands suggesting the presence of a centromere by CBG-banding.
somes, a mechanism for dosage compensation between females and males has evolved in order to achieve genetic equivalency between the two. The X chromosome inactivation is a developmentally regulated process by which dosage compensation occurs and is governed by the X inactivation center which contains the XIST gene. Mental retardation, severe short stature, and other malformations are not normally associated with TS patients having a 45,X karyotype. These atypical features in TS correlate with the presence of small r(X) chromosomes and may be due to the absence or malfunction of the XIST gene.\textsuperscript{19,20} These alterations are likely to result in the inappropriate expression of X chromosome loci that should normally be transcriptionally inactive.

Results from our study are consistent with the observations that SMCs associated with unusual TS phenotypes tend to be small, annular in structure, and X chromosome derived. Further studies of fibroblast cultures with cells having two r(X) chromosomes derived from our patient* have shown: (1) the presence of XIST sequences in the larger ring, but the absence of XIST sequences in the smaller ring by FISH analysis; and (2) XIST expression by molecular methods. These results indicate that in the cell line having the two r(X) chromosomes in fibroblasts, one ring indeed inactivates normally (and expresses XIST), but the second ring appears to be activated and not to contain sequences homologous to the XIST locus.\textsuperscript{†} Therefore, the likely explanation for the severe phenotype in our patient is that other tissue types may contain r(X)s in which the XIST may be missing or malfunctioning, and that this defect may be limited to a particular tissue type.

The absence of the XIST transcript (either because the gene is missing or because of regulatory dysfunction) may represent a general mechanism which results in an altered pattern of dosage

\* In laboratory of Dr. B. R. Migeon, Johns Hopkins University, Baltimore, MD.
\textsuperscript{†} Jani et al: manuscript in preparation.
compensation having unexpected clinical consequences. Further investigations of these small r(X) chromosomes may lead to a long awaited, better understanding of the genetic mechanisms regulating dosage compensation in mammals and may also provide a better idea of how aberrant X chromosome inactivation may be related to specific human pathologies.

References