

TURNER SYNDROME

Molecular identification of chromosome Y sequences in Brazilian patients with Turner syndrome

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Abstract

The investigation of Y-specific sequences in patients with Turner Syndrome (TS) with karyotype 45,X or mosaic, has a fundamental role in the clinical management of these patients. The relationship between the presence of Y chromosome fragments and a higher risk of gonadoblastoma in TS has already been established. The aim of the study was to investigate the presence of Y-chromosome fragments in a population of 42 female Brazilian patients with TS from Mato Grosso state. Cytogenetic analysis has shown the karyotypes 45,X in 27 of them (64.3%) and mosaic in 15 (35.7%). The presence of the Y-primers SRY, DYZ3, ZFY, DYZ1, DYS1 and PABY was investigated in all patients. These markers were amplified by polymerase chain reaction (PCR) technique, using DNA genomic from peripheral blood lymphocytes. None of these patients had shown any Y-chromosome fragments when they were analysed only by the classic cytogenetic technique. The PCR analysis with the Y-specific sequences ZFY and DYZ3 were identified in two different patients (4.8%), both with karyotype 45,X. It was concluded that PCR is efficient in the investigation of hidden Y-fragments in TS patients. Therefore, this method should be included in the routine assistance of these patients.

Keywords: Turner syndrome, Y-specific sequences, gonadoblastoma

Introduction

The presence of Y-chromosome fragments in patients with Turner's syndrome (TS) is associated with the increased risk of development of the benign tumor gonadoblastoma, which has considerable malignant potential. By standard cytogenetic analysis, ~6% of patients with TS have a Y chromosome and an estimated 3% carry an unidentified marker chromosome [1,2].

TS is a chromosomal abnormality present in 1:2500 live-born women. This condition is responsible for about 20% of all cases of spontaneous abortion. The phenotype of TS patients is variable, including short stature, gonadal dysgenesis, uncommon typical facies, low posterior hair line and lymphedema. In 50–60% of them, the karyotype shows an X chromosome monosomy (45,X) and the remaining cases present either X or Y structural abnormalities [3]. As a number of genes are involved in the process of the

gonadal differentiation, any alteration in the mechanisms involved in this process can result in a disgenetic gonad, as that verified in TS patients [4,5].

Because gonadoblastoma can be expressed as a function of the presence of the locus GBY on a Y chromosome, the existence of a Y chromosome or Y-derivative material in TS may contribute to the development of this neoplasia in their disgenetic gonad [4]. Conventional chromosomal analysis indicates that 4–20% of patients with TS may have a Y chromosome or Y-fragments. This percentage can be even higher, since the more sensitive polymerase chain reaction (PCR) analysis was introduced [3,5,6]. When PCR was used for identification of Y chromosome sequences in different tissues of TS patients, positive results have been found in 0–60% of them [5–7]. Thus, this molecular approach allows identifying TS patients with higher risk of gonadoblastoma development. In the current study, the presence of the Y chromosome sequences SRY, DYS1, DYZ1,

PABY, ZFY and DYZ3 was investigated in TS Brazilian women.

Material and methods

This cross-sectional study includes 42 subjects between 1 month and 40 years of age (mean \pm SD, 14.9 ± 2.4 years old) in whom the clinical diagnosis of TS was confirmed by karyotype. The karyotype distribution is presented in Table I. Patients were recruited from the Public Health Service of Mato Grosso State, Brazil. The equation for sample size was: $n = (1.62)^2 \times p(1-p)/\delta^2$, p = estimated prevalence of Y-chromosome fragments in 3% TS patients, confidence interval of 95%, and δ = imprecision of 6%. The study protocol was approved by the local ethics committee and all patients and/or their parents gave informed consent before starting the study.

Cytogenetic studies were performed in peripheral blood lymphocytes. At least 30 metaphases were analysed in each patient. The lymphocyte cells were cultivated for 72 h with RPMI 1640 medium supplemented with 10% calf serum and phytohemagglutinin (Cultilab[®], EUA). The chromosome preparations were carried out according to the technique proposed by Moorhead et al., with slight modifications [8]. Briefly, 5 mL of RPMI 1640 (Difco[®], USA) containing 20% of fetal calf serum, 0.2 mL phytohemagglutinin and 0.4 mL of blood were incubated at 37°C for 72 h. Mitosis was stopped by adding 0.1 mL of colchicine (Sigma[®], Brazil) to the culture mixture.

Genomic DNA was extracted and screened for Y chromosome in all participants through molecular studies. The protocol described by Lahiri and Numberger was used with slight modifications [9]. Six different sets of primers, encompassing both arms of the Y chromosome, SRY, DYZ3, ZFY, DYS1, DYZ1 and PABY were used (Figure 1). PCR was performed in a final reaction volume of 25 μ L, containing 1.5 μ g of genomic DNA, 1.5 mM of MgCl₂, 0.02 mM of each dNTP, 0.05 μ M of each primer and 2.5 U of Taq DNA polymerase (Invitrogen[®], USA), for each of the six sets of primers. Amplification was carried out in a thermal cycler Gene Amp PCR System 9600, Perkin Elmer[®]. To

avoid false-positive results, internal controls were used, and all samples were processed by the same operator. Healthy male DNA and a blank reaction were used as control for all runs. A rhodopsin gene amplification was performed as DNA quality control for the experiments. The products of PCR were submitted to electrophoresis on 1% agarose gel, stained with ethidium bromide, observed under UV light and photographed. The samples of each patient included in this study were tested by PCR for three times to confirm the results; also, these same samples were sent to a reference service in the Genetic Division, Morphology and Genetics Department and Endocrinology Division, Medicine Department/ Universidade Federal de São Paulo, Brazil.

Results

Fragments for Y-chromosome of 42 TS patients were investigated by classic cytogenetic technique and PCR. The cytogenetic analysis has showed 45,X in 27 patients (64.2%) and 15 (35.8%) with other karyotype composition (Table I). The PCR approach was used to investigate the presence of Y-specific sequences, disclosed hidden mosaicism in two patients (4.8%). These results are summarised in Table II. The Y-fragment DYZ3 (Figure 2) was observed in one patient and the Y-sequence ZFY in another one (Figure 3), both with karyotype 45,X. Clinically, the patient with ZFY sequence presented a small jaw, palate in ogivae, overlapping teeth, spina bifida, acute otitis, heart disease, excessive shyness and difficulties in school performance. The patient with DYZ3 positive sequence presented a tendency to keloids, a narrow jaw, overlapping teeth, spina

Table I. Distribution of the karyotype observed in 42 patients with Turner syndrome.

Karyotype	n	(%)
45,X	27	64.28
45,X/46,XX	11	26.20
45,X/46,Xi(Xq)	3	7.14
45,X/46,X,del(Xq)	1	2.38
Total	42	100

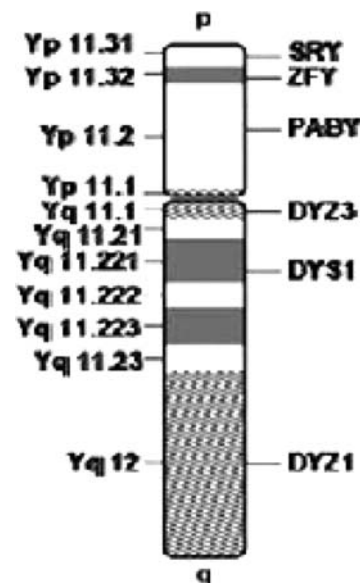


Figure 1. Ideogram showing the six regions of the Y chromosome locations analysed in this study.

Table II. Y-specific sequences detected in two patients with Turner syndrome.

Patients	Age	Karyotype	Y-specific sequences analysed					
			<i>SRY</i>	<i>ZFY</i>	<i>DYZ1</i>	<i>PABY</i>	<i>DYS1</i>	<i>DYZ3</i>
N.M.M	17	45,X	-	-	-	-	-	+
P.G.M	08	45,X	-	+	-	-	-	-

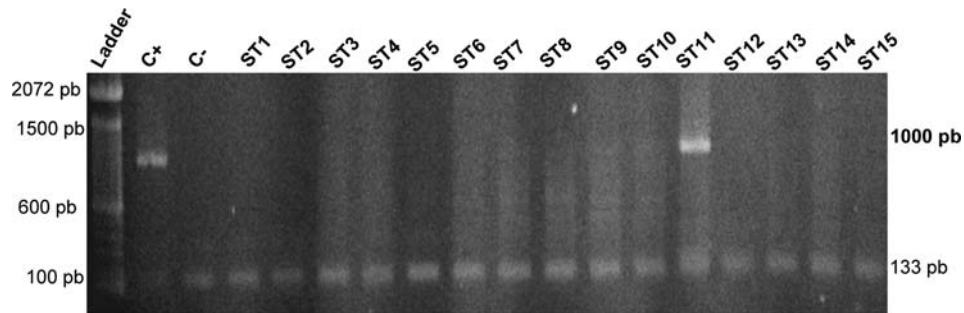


Figure 2. Agarose gel electrophoresis of the PCR products of the patients TS1 to TS15 for analysis of the *DYZ3* fragment. Patient ST11 shows positive result for this fragment. Line 1 shows standard molecular weights, lines 2 and 3 positive (C+) and negative (C-) controls. The first gene of rhodopsin (133 pb), shown on all lines was used as control of the DNA quality in all reactions.

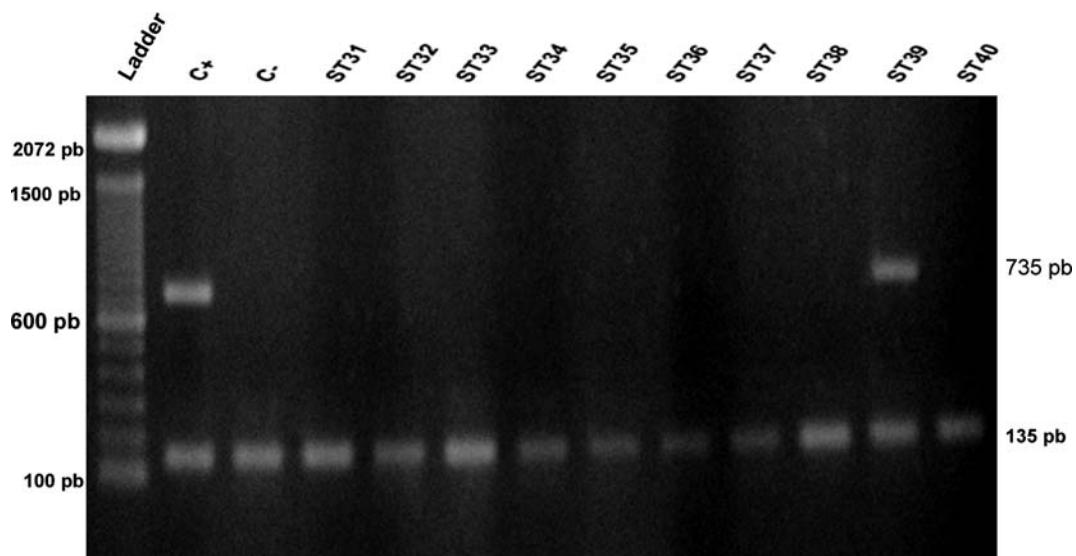


Figure 3. Agarose gel electrophoresis of the PCR products of the patients ST31 to ST40 for analysis of the *ZFY* fragment. Patient ST39 shows positive result for this fragment. Line 1 shows standard molecular weights, lines 2 and 3 positive (C+) and negative (C-) controls. The first gene of rhodopsin (133 pb), shown on all lines was used as control of the DNA quality in all reactions.

bifida, feet deformity, acute otitis, a horseshoe kidney and great difficulty in school performance. None of them had any clinical sign of hyperandrogenism. For two patients with positive results to Y-specific sequences were conducted to have preventive gonadectomy and are being observed by a multidisciplinary team, with biologist, psychologists, geneticists, endocrinologist and gynecologist.

Discussion

The X-chromosome monosomy is the main chromosome disorder found in TS patients. In addition to this

karyotype, a broad range of other karyotypes can be found. These chromosome disorder varieties in TS patients contribute to their different clinical and phenotypical aspects such as: clinical differences from severe short stature, gonadal dysgenesis, lymphedema and somatic dysmorphic features of women with only a slight reduction in the final height or premature ovarian failure [1,5,7,10,11]. In the current study, the data are consistent with the majority of the cytogenetic studies including TS patients worldwide.

Molecular analysis has been increasingly used as a fundamental tool in the evaluation of TS patients because these patients have increased risk to develop

gonadal tumors, especially gonadoblastoma, when Y-chromosome fragments are present. It is estimated that ~90% of patients with gonadoblastoma have Y-chromosome material in their genetic constitution. In Brazil, Y-chromosome sequences in TS patients have been reported to range between 3.0 and 8.0% [3,12]. In the current study, using molecular analysis with six different Y primers chromosome, the presence of Y-sequences was detected in two of them (4.8%), both with karyotype 45,X. This result is consistent with most studies conducted with this objective in other countries [1,6,10,11,13].

A previously described in the literature, PCR is more sensitive than karyotyping in detecting hidden Y chromosome material in patients with TS. The incidence of Y-chromosome sequences in patients with TS has been evaluated in several studies [12]. The use of PCR or FISH to investigate Y-chromosome sequences in patients with TS in several countries has shown a prevalence varying between 2.5 and 16.6% (Table III).

In Latin-America, a few studies have been performed. In Mexico City, a study including 107 TS patients with karyotype 45,X analysed the possible presence of the fragments SRY, ZFY and PABY. In 10 of them, at least one of these fragments was present (9.3%). Later, in two of them the diagnosis of gonadal tumor was confirmed [7]. A search for the Y-sequences, ZFY and SRY, in another study, 50 Mexican karyotype 45,X patients showed that these sequences were present in four of them (8%) [13]. The search for the sequences ZFY, DYZ3, DYS19 and TSPY showed a prevalence of 7.6% of Y-fragments in 52 Venezuelan TS patients with karyotype 45,X [19]. In Chile, the sequences SRY, TSPY and DYZ3 were identified in four of 58 (6.8%) TS patients with a karyotype 45,X or mosaic [1].

In Brazil, three studies have reported on the presence of Y-fragments in patients with TS. A study conducted in the city of São Paulo, including 122 patients with karyotype 45,X or mosaic, has investigated the presence of eight Y-sequences. In four of them (3.3%) at least one Y-fragment was detected [12]. The analysis of the Y-sequences SRY, ZFY and

DYZ3 in another 36 Brazilian patients with TS identified the presence of at least one of these fragments in two of them (8.3%) [3]. In another study in São Paulo city, including 20 patients with TS and karyotype 45,X, the sequences SRY and DYZ3 were investigated in three different tissues. In six (30%) of these patients, the marker SRY was found and in four (20%) of them the sequence DYZ3 was present [5].

Looking at only the studies in which more than one tissue was analysed, the prevalence of Y sequences has been reported to vary between 2.3 and 60% [4,5,10]. When the tissue examined was only peripheral blood, 3–20% of TS patients have shown the presence of Y-fragment. Among the studies including Y-material analysis in cells of the oral mucosa, the prevalence of Y-fragment ranged from 0 to 42% [1,6,11,13,14,18,19].

The SRY and DYZ3 sequences are the Y-fragments most frequently used. In general, the SRY gene sequences are used as reference because of their localising importance in the signalling cascade of sex-determining events [5]. This gene is the most investigated one, and its detection is almost unanimous, its detection is considered essential in studies including TS patients with mosaicism. However, with the possible identification of new Y-chromosome genes specific to predict gonadoblastoma, other markers will soon be included in the clinical setting [3–5,14].

When the number of primers included in the analysis is considered, the prevalence of Y-fragments still varies with the population analysed. When two primers were analysed, the prevalence found ranged between 0 and 42% and with the inclusion of three primers it was 2.5–60%; when more than three primers were looked at, the prevalence found was 2.5–100% [1,3,6,11,13,15,16].

Although the prevalence of the Y chromosome sequences in patients with TS has been frequently estimated to range between 2.3 and 15%, the real frequency still depends on the technique and tissue included in the analysis. In synthesis, if all studies are considered, the prevalence may range from 0 to 60% [5]. Several factors contribute to this difference in the findings, such as: sample size, patient selection, methodology and primers used in different studies might explain so large a prevalence variation. It is concluded that the investigation of Y-sequences in patients with TS has an important role in establishing the most appropriate therapeutic approach for these patients, so that patients with higher risk to develop gonadoblastoma are identified early. However, the efficacy in detecting the true prevalence still depends on many variables.

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Table III. Prevalence of Y-specific sequences in TS patients in different countries.

Population studied	Number of patients with Y-specific sequences	Prevalence of Y-specific sequences (%)	Reference
France	1/40	2.5	6
Australian	6/208	3	14
England	2/50	4	15
Turkey	2/40	5	11
Italian	14/171	8	16
Denmark	14/114	12.2	4
Denmark	6/40	15	17
Russian	5/30	16.6	18

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