

Y-Chromosome Detection in Turner Syndrome

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Abstract

Turner syndrome is a chromosomal disorder characterized by the presence of a single normal X chromosome in women. Additionally to the X chromosome monosomy, other cell lines can co-exist, containing the Y chromosome or part of it. The presence of Y chromosome in patients with Turner syndrome represents an increased risk (15-30%) of developing gonadoblastoma. In this study we screened for the absence/presence of four genes mapped on Y chromosome (SRY, TSPY, DDX3Y and HSFY) in 98 female samples obtained from different tissues, namely peripheral blood, amniotic fluid, gonadal tissue and miscarriages samples, previously characterized cytogenetically having at least one cell line with monosomy X or an abnormal X chromosome. We also evaluate the importance of a molecular test for detection of Y chromosome sequences using a combination of conventional cytogenetic methods and DNA analysis. Three miscarriages and one gonadal tissue presented Y-chromosome DNA sequences out of the 98 studied samples. We have discussed the higher frequency of the Y sequences in spontaneous abortions with 45, X karyotype and we have advised the detection of Y-chromosome material in Turner patients in order to improve the clinical orientation and the consequent prognosis.

Keywords: Turner syndrome (TS); 45, X; Gonadoblastoma; Y chromosome sequences; PCR

Introduction

Turner Syndrome (TS) with karyotype 45, X is one of the most common cytogenetic abnormalities (1:2500 among newborn females). It is compatible with life, even though the great majority (more than 99%) of the conceptuses with karyotype 45, X are a spontaneously loss, usually before 28 weeks of gestation [1].

Individuals with TS, they present extremely variable phenotypes. Despite their undifferentiated gonads, women usually have clearly female external and internal genitals. However, the uterus normally is small and its development depends on hormonal stimuli. Almost all girls with TS have short stature (98%) and ovarian failure. TS is characterized by a multisystemic involvement (cardiac, renal, orthopedic, ophthalmic and physical), responsible for a high morbidity and an increased mortality at all ages [2].

Cytogenetically, the TS is characterized by sex chromosome monosomy (45, X) in phenotypically female individuals and this karyotype is found in 50-60% of the cases. The remaining cases are mosaics with a 45, X cell line plus a normal line (46, XX), 47, XXX and/or structural anomalies (isochromosomes of the long arm, dicentric chromosomes, deletion of the short arm or ring chromosomes). This is found in ~30% of the cases [3]. Finally, mosaicism with a cell line presenting a normal or abnormal Y chromosome (isochromosome of the long arm and dicentric chromosomes) is identified in 6-11% of patients with TS [4]. However, it has been suggested that cryptic mosaicism, for at least part of the Y-chromosome, may be present more frequently [5]. Thereby, accurate detection of the Y chromosome or its fragments is clinically important because some studies have reported that almost 40% of TS patients develop gonadoblastoma [6,7]. Gonadoblastoma is a type of germ cell-sex cord-stromal tumor, consisting of neoplastic germ cells and sex cord-stromal derivatives. It typically arises in the streak gonad of patients with pure or mixed gonadal dysgenesis, the minority of whom are phenotypic male with varying levels of feminization and the great majorities are the young phenotypic females with amenorrhea or virilization. The association of the Y chromosome and gonadoblastoma has been well established and the Y chromosome has an important role in tumorigenesis [8]. The

standard cytogenetic techniques may not detect structurally abnormal chromosomes especially if these abnormalities are very small or in very low frequency and more reliable techniques are needed to improve an accurate diagnose. The Polymerase Chain Reaction (PCR) technique has allowed a sensitive study of TS patients by revealing the existence of hidden mosaics which are not detected at the cytogenetic analysis [6,9].

The aim of this study was to check the absence/presence of four genes mapped on Y chromosome, in 98 cases (TS patients or miscarriages with 45, X) and evaluate the importance of a molecular test for detection of Y chromosome sequences, which escape to detection by conventional cytogenetic techniques. The genes studied were SRY (Sex Determining Region on the Y chromosome), TSPY (Testis-Specific Protein, Y-encoded), DDX3Y (DEAD/H box polypeptide, Y-chromosome) and HSFY (Heat Shock Transcription Factor, Y chromosome). These genes are mapped in different regions of the Y chromosome. In general, the SRY gene is the most investigated sequence because it has an important role in the sex differentiation cascade and due to its location [3]. The detection of this gene is almost unanimously considered of major importance when studying mosaicism in TS [10]. The TSPY is one of the candidate loci for gonadoblastoma, a multicopy gene that is located in the GBY critical region. The TSPY expression has been detected in gonadoblastoma tissues [10]. The DDX3Y gene is thought to be the major Azoospermia Factor (AZFa) gene functionally required for male fertility as its deletion was found in men with the SCOS (Sertoli-Cell-Only-Syndrome) [11]. HSFY has been mapped in the AZFb region

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Received September 16, 2013; **Accepted** November 16, 2013; **Published** November 20, 2013

Citation: Fernandes S, Ventura V, Dória S, Barros A (2013) Y-Chromosome Detection in Turner Syndrome. Human Genet Embryol 3: 115. doi:10.4172/2161-0436.1000115

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of the Y chromosome, whose deletion is responsible for severe male infertility. This gene belongs to the HSF (Heat Shock Factor) family that has been shown to be implicated in spermatogenesis both in animals and humans [12].

Material and Methods

Patients

Peripheral blood samples of 81 individuals, 2 samples of amniotic fluid, 1 gonadal tissue and 14 miscarriages samples were included in this study. The age range was 3-58 years. The indications for postnatal study included the most common alterations associated with this syndrome (short stature, gonadal dysgenesis with primary amenorrhea, poor development puberty and infertility). The indications for prenatal study were cystic hygroma, increased nuchal translucency, maternal triple screening test and intra uterine growth restriction. The main indications for cytogenetic miscarriages studies were cystic hygroma and recurrent abortions. Informed consent was obtained from all participants (Genetic Testing and Personal Data Protection National Laws).

Methods

Cytogenetic analysis: Karyotyping was performed from peripheral blood lymphocyte, fibroblasts cells from the amniotic fluid and the miscarriages samples cultures using GTL-banding technique. On average, 20-30 metaphases per patients were counted.

DNA extraction: The genomic DNA was extracted from peripheral blood samples or cells fixed in methanol: acetic acid using a commercial kit (Blood & Cell Culture DNA Spin Kit – Genomed). Additionally, for the DNA extraction from the amniotic fluid and miscarriages samples a commercial kit for purifying DNA (ReadyAMPTM Genomic DNA Purification System – Promega Corporation) was used.

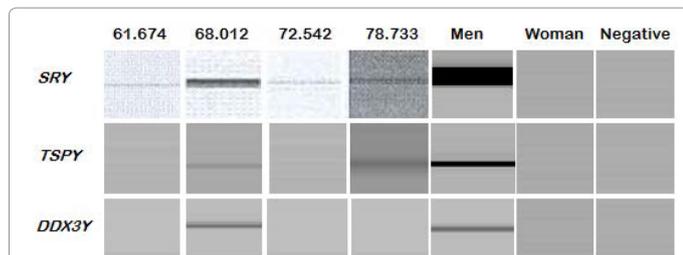


Figure 1: PCR amplification of SRY, TSPY and DDX3Y fragments in positive patients and male and negative controls (a female DNA and a negative control for PCR - with all reagents but DNA).

DNA analysis: Four genes (SRY, TSPY, DDX3Y and HSFY) were analysed by PCR. Amelogenin gene (AMXY) is a single copy gene present in both sex chromosomes and it was additionally used as a gene control.

All PCR were performed in a final reaction volume of 25 µl, containing 1-3 µl of DNA; 2.5 µl of PCR Gold Buffer; 1.5 mM of MgCl₂; 2.5 mM of each deoxyribonucleotide triphosphate; 12.5 µmol/µl of each primer and 0.3 µl of Amplitaq Gold (Applied Biosystems). Amplification was performed in a thermal cycler (Biometra T3000) and consisted of a 5-minute denaturing step at 95°C, followed by 35 cycles of 1 minute at 94°C (denaturing), 1 minute of annealing (58-60°C) and 1 m 30 sec at 72°C (extension). At the end final extensions step of 10 minutes at 72°C. The primers sequences used and the respective annealing temperatures are indicated on (Table 1).

The reaction products were analyzed on a multicapillary electrophoresis instrument QI Axcel (QIAGEN).

After testing the dilution series of a genomic DNA sample we were able to detect Y-chromosome fragments using DNA amounts ≥ 100 ng.

Results

Cytogenetics

All cases were karyotyped. The cytogenetic findings of the 81 peripheral blood samples are summarized on (Table 2). All miscarriage or amniotic fluid samples and the gonadal tissue presented a 45, X karyotype.

Y chromosome sequences study

The study of the presence or absence of SRY, TSPY, DDX3Y and HSFY genes was performed by PCR in all 98 samples. From 14 miscarriages samples we had 3 positive with the presence of the SRY and TSPY gene (61674, 72542 and 78733). The gonadal tissue sample was positive for SRY, TSPY and DDX3Y genes (68012). All the peripheral blood samples were negative for the genes studied. A frequency of 4.08% (4/98) positive samples was found indicating the existence of Y chromosome material at least in a minority cell line (Figure 1). The results are summarized on Table 3.

Discussion

In this study Y chromosome material was identified in 4 (4, 08%) of 98 cases (24 patients with pure monosomy X and 74 patients with a line 45, X plus a normal line (46, XX) or abnormal lines).

Previous studies have found frequencies of hidden Y chromosome

Gene	Primer sequence (5'→3')		Product length (bp)	Annealing temperature
AMXY	F AMXY	5'-6-FAM-CTG ATG GTT GGC CTC AAG CCT-3'	432	58°C
	R AMXY	5'-GGT GGC GGC TGT TGC TGC TG-3'		
SRY	F sY14	5'-GAA TAT TCC CGC TCT CCG GA-3'	472	60°C
	R sY14	5'-GCT GGT GCT CCA TTC TTG AG-3'		
TSPY	F TSPY	5'-CCT TTC ATC CCA ACC TTT ATT TCC A-3'	273	62°C
	R TSPY	5'-GCA GTC ATG TTC AGC CAAACA GC-3'		
DDX3Y	F DBY	5'-ATC GAC AAA GTA GTG GTT CC-3'	688	58°C
	R DBY	5'-AGA TTC AGT TGC CCC ACC AG3'		
HSFY	F sY114	5'-TGC ACT CAT GGA GAC AAC AG-3'	1450	60°C
	R sY114	5'-AAC CAG GGT TTT CAC TGAAA-3'		

Table 1: Sequences of the primers used in four PCR reactions and their annealing temperatures [19-22].

No. of cases	Biological samples	Karyotype	% mosaicism	Mean age of each group	Clinical information
7	Pb	45,X	0	7.4	Clinical suspicion of TS
33	Pb	45,X/46,XX	5-10%	33.5	Infertility /primary amenorrhea / gonadal dysgenesis
1	Pb	45,X/46,XX	n.a.	n.a.	Diagnosis unknown
19	Pb	45,X/46,XX	>10%	32.5	Infertility / short stature
7	Pb	45,X/47,XXX/46,XX	5-10%	35	Infertility
1	Pb	45,X/47,XXX/46,XX	n.a.	n.a.	Diagnosis unknown
4	Pb	45,X/47,XXX/46,XX	>10%	38	Infertility / poor development puberty
1	Pb	45,X,inv(21)(p12q22.1)/47,XXX,inv(21)(p12q22.1)/46,XX,inv(21)(p12q22.1)	13,70%	36	Family history with trisomy 21
1	Pb	45,X/46,X,der(X)	0	11	Clinical suspicion of TS
1	Pb	46,X,inv(X)(p22.1q13)	0	29	Infertility
1	Pb	46,X,?dup(X)(q25q27)	0	10	Diagnosis unknown
1	Pb	46,X,?dup(X)(q26)	0	38	Difficulty in learning
1	Pb	46,X,del(X)(p11.2)	0	11	Short stature
2	Pb	46,X,del(X)(q24)	0	32.5	Infertility
1	Pb	46,X,inv dup del (X) (qter --->q24 :: p22.1 --->qter)	0	34	Infertility
2	Af	45,X	0	34	Cystic hygroma / Increased nuchal translucency / maternal triple screening / intra uterine growth restriction
3	Ft	45,X	0	30.5	Fetal death after 12 weeks gestation
1	P	45,X	0	27	Cystic hygroma
1	Gt	45,X	0	3	Gonadal dysgenesis
10	Of	45,X	0	33.4	Recurrent abortions

Pb: Peripheral blood; AF: Amniotic Fluid; Ft: Fetal tissue; P: Placent; Gt: Gonadal tissue; Of: Ovular fragments

Table 2: Biological samples studied and their karyotype

Number	Biological sample	Karyotype	SRY	TSPY	DDX3Y	HSFY
61674	Ovular fragments	45,X	+	-	-	-
68012	Gonadal tissue	45,X	+	+	+	-
72542	Ovular fragments	45,X	+	-	-	-
78733	Ovular fragments	45,X	+	+	-	-
			4/98	2/98	1/98	0/98

Table 3: Positive results in the different samples.

material ranging from 0% to 60% [10]. In 1992, Medlej et al. studied 40 patients (37 patients with 45, X and 3 patients with 45, X/46, XX) and found one SRY positive case among the 45,X cases [13]. Held et al. studied 87 patients (18 patients with 45, X, 42 patients with mosaicism, 16 patients with mosaicism plus a second cell line containing a small marker chromosome and 11 patients with a structural aberration of the X chromosome). From that, 8 patients showed additionally the presence of Y material [14]. In 1995, Coto et al. found in 18 patients (15 patients with 45,X and 3 patients with 45,X/45,Xmar+) frequencies of SRY material of 27.7% [15]. López et al. in 1998 studied 50 patients with TS using PCR and observed that 24% and 12% of the 45, X patients with or without mosaicism respectively, presented Y chromosome specific sequences [9]. Nazarenko et al. studied 50 females with a clinical suspicion of TS using PCR (primers DYZ3 and DYZ) and the results were negative for all patients [16]. More recently, Bianco et al. detected SRY sequence in 3 of the 5 patients studied with Y-chromosome mosaicism. The patients were selected on the basis of the presence of karyotype abnormalities such as marker chromosome, additional material, or ring chromosomes [17]. Comparative studies are difficult as each study used a different criterion for the selection of patients. Additionally, the methods and the markers selected to study the Y chromosome sequences also contributed for the discrepancies found in the different studies which were reported.

If we consider the results obtained in our study [16.7% for 45, X samples (4/24) and 4.08% for all samples analysed (4/98)], we could consider that the main difference reported is mainly due to the total of samples studied and their karyotypes. For this study, samples were selected only from patients with X chromosome monosomy or mosaic karyotype, wherein in addition to the 45, X cell line there are one or more cell lines with the two or more X chromosomes with normal or abnormal structure. Therefore, all the patients with cytogenetic evidence of Y chromosome material were excluded and this was a factor that contributed for the increased values obtained in the majority of other studies.

In another hand, it is important to stress that 3 of the 14 (21.4%) miscarriages samples with full 45, X karyotype were positive for Y sequences. Therefore, as far as we understand, this is the first study reporting the presence of Y chromosome regions in miscarriages samples with karyotype 45, X. As we mentioned before, it was recognized that the frequency of Y chromosome material was high depending of the inclusion criteria of the studies, namely our study suggest that the frequency could be higher in 45, X spontaneous abortions. More studies with large series should be performed to confirm this finding.

We already know that the majority of the X monosomy conceptions end in a spontaneous abortion being in 80% of these cases the paternal

chromosome absent. Although 45, X is quite lethal in fetus, those that survive to term have relatively minor problems. The reason for this has been speculated using the argument that all the conceptions that survive should have some degree of undetected mosaicism for a normal cell line. In another hand, and taking into account the possibility of a higher frequency of Y sequences in 45, X miscarriages samples comparing with peripheral blood samples we speculate if the presence of Y chromosome sequences could contribute for the severity of the phenotype and increase the possibility of a fetal loss.

In respect to the gonadal tissue, the patient present a karyotype 45, X and expressed some of the typical characteristics of the TS (short stature, delay in the expression (QI 71- normal slow) and female external and internal genitals). The molecular studies revealed the presence of SRY, TSPY and DDX3Y genes in the gonadal tissue. A bilateral gonadectomy was performed according with clinical criteria to reduce the possibility of malignant degeneration.

The question of which TS patients should be tested at the DNA level deserves further consideration. Classical cytogenetic technique do not detected the majority of mosaics 45, X if the percentages of the abnormal or monosomic cell line is underrepresented (low grade mosaicism), since up to 60 cells (maximum) are normally counted in a routine cytogenetic study. So, the mosaicism below 5% could not be detected. The PCR tests allow a more detailed study of these patients. In a recent study including 52 cytogenetically diagnosed 45, X patients, two gonadoblastomas were found, and the present of Y-chromosome material was confirmed by PCR in both cases [18]. Overall, we suggest the identification of Y-chromosome material in patients with 45, X karyotype who had neither virilization nor marker chromosome positivity.

As an overall conclusion, the use of PCR technique for TS diagnosis could be a complementary diagnostic method contributing to a more accurate clinical diagnosis, helping to reduce the morbidity and to improve the life quality and expectancy of these patients. More studies in miscarriages with 45, X karyotype should also be performed in order to confirm our hypothesis of an association between the presence of Y-chromosome sequences and a higher lethality of 45, X chromosome constitutions.

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