Original article

Y chromosome in Turner syndrome and FISH technique usefulness in cytogenetic diagnosis

Cromozomul Y in sindromul Turner şi utilitatea tehnicii FISH în diagnosticul citogenetic

Diana-Laura Miclea[1*], Paula Grigorescu-Sido[2], Simona Bucerzan[2], Ioan Victor Pop[1]

1. Molecular Science Department, Medical Genetics Chair, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania
2. Mother and Child Department, Children's Emergency Hospital Cluj, Medical Genetics Department, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania

Abstract

Context. Y chromosome detection in Turner syndrome (TS) has a particular clinical significance because it involves up to 30% risk for gonadoblastoma development. Using standard cytogenetics techniques, Y chromosome is observed in approximately 6% of studied cases. Using FISH techniques (Fluorescent in Situ Hybridization) an additional number of cases initially undetected by conventional cytogenetics with chromosome Y has been diagnosed. The management of these situations is prophylactic gonadectomy. Aims. The principal aim of the study was Y chromosome detection using FISH technique in patients with TS. Other sex chromosome abnormalities were assessed within the same analysis. Material and method. 21 patients with TS were included in the study. The patients standard karyotypes were homogeneous monosomy 45,X in 13 cases and mosaicism in 8 cases, with a second cell line, of which: 2 patients - 46,XX, 2 patients- 46,Xi(Xq), one patient – 46,X,r(X), one patient – 46,X,del(Xp) and 2 patients – 46,X,+mar. FISH technique was used to analyze sex chromosomes abnormalities. Results. FISH technique failed to reveal the Y chromosome in these patients. FISH technique allowed the marker chromosomes identification as originating from the X chromosome. Also, in two patients diagnosed with homogeneous monosomy 45,X by conventional cytogenetics, the FISH technique pointed out a low-level mosaicism. Conclusion. By comparing with literature data, we have highlighted the utility of FISH technique, as a sensitive, specific and fast quantitative technique for the detection of Y chromosome, also for chromosome marker identification and low level mosaicism detection involving X chromosome in TS patients.

Keywords: *Turner syndrome, Y chromosome, mosaicism, FISH*

[*]**Corresponding author**: Diana-Laura Miclea, Molecular Science Department, Medical Genetics Chair, University of Medicine and Pharmacy "Iuliu Hatieganu", 8, Victor Babes street, 400012, Cluj-Napoca, Romania. Phone: 0040264597794 ; E-mail: bolca12diana@yahoo.com; bolca.diana@umfcluj.ro

Rezumat

Introducere. Detecția cromozomului Y în sindromul Turner (ST) este deosebit de importantă pe plan clinic, întrucât implică un risc de până la 30% de dezvoltare a gonadoblastomului. Prin efectuarea cariotipului standard, se observă prezența cromozomului Y la aproximativ 6% din paciente. Folosirea tehnicilor FISH (Fluorescent in Situ Hybridisation) conduce la evidențierea unui număr suplimentar de cazuri care prezintă cromozom Y, nedetectate inițial prin citogenetica convențională. Conduita terapeutică în aceste situații este efectuarea gonadectomiei profilactice. Obiective. Scopul principal al studiului a fost detecția cromozomului Y la pacientele cu ST, folosind tehnica FISH. Aplicând aceasta tehnică, au fost evaluate şi alte anomalii ale cromozomilor sexuali. Material şi metodă. în studiu au fost incluse 21 paciente cu ST. Cariotipurile acestor paciente au fost reprezentate de monosomie omogenă 45,X în 13 cazuri şi mozaicisme în 8 cazuri, cu o a doua linie celulară, astfel: 2 paciente- 46,XX, 2 paciente– 46,X,i(Xq), o pacientă - 46,X,r(X), o pacientă - 46,X,del(Xp) şi 2 paciente- 46,X,+mar. Pentru analiza cromozomilor sexuali s-a folosit tehnica FISH. Rezultate. Folosirea tehnicii FISH nu a evidențiat cromozomul Y la aceste paciente. Tehnica FISH a permis determinarea originii cromozomilor marker, ca provenind din cromozomul X. De asemenea, la două paciente diagnosticate cu monosomie omogenă 45,X prin citogenetică convențională s-a observat, în urma aplicării tehnicii FISH, prezența unui mozaicism de nivel scăzut. Concluzii. Comparând cu datele din literatura de specialitate, am pus în evidență utilitatea tehnicii FISH, ca tehnică cantitativă sensibilă, specifică şi rapidă de evidențiere a cromozomului Y şi în plus de determinare a originii cromozomilor marker şi de evidențiere a mozaicismelor de nivel scăzut implicând cromozomul X în ST.

Cuvinte cheie: *sindromul Turner, cromozomul Y, mozaicism, FISH*

Received: *19th September 2012;* ***Accepted:*** *8th November 2012;* ***Published:*** *15th December 2012.*

Introduction

Turner syndrome (TS) is defined as complete or partial absence of the second sex chromosome, with or without the presence of cell mosaicism and is seen in one in 2500 female newborns (1, 2). Positive diagnosis is established by performing standard karyotype, ascertaining the presence of the Y chromosome (normal or with structural abnormalities) in approximately 6% of analyzed cases, by using molecular techniques this percentage is higher (3).

Y chromosome detection in TS is particularly important from a clinical point of view, as it involves up to a 30% risk of gonadoblastoma development (4, 5). Gonadoblastoma is an *in situ* cancer of the germs cells and has the potential to progress to invasive cancers, often dysgerminoma and less frequently other tumours such as embryonic carcinoma, teratoma, yolk sac tumor and choriocarcinoma (6). 60% of cases with gonadoblastoma progress to dysgerminoma development (7). The critical region for gonadoblastoma is presumed to be located in a pericentromeric small region on the Y chromosome short arm (GBY - gonadoblastoma locus on Y) (8). Management when Y chromosome sequences are found in TS is prophylactic gonadectomy.

FISH (fluorescent *in situ* hybridization) and PCR (polymerase chain reaction) techniques allow the detection of Y chromosome in previously undiagnosed cases by conventional cytogenetics (9, 10). Y chromosome is recommended to be searched by molecular techniques in TS in any patient showing virilizing signs or a marker chromosome in standard karyotype (1). It is not known if a patient without marker chromosome or virilization should be investigated for Y chromosome and therefore additional studies are required in this respect.

FISH technique is usually recommended in the detection of the Y chromosome, as PCR methods are sometimes associated with an increased rate of false positive results (1, 11).

The aim of this study was to investigate the presence of the Y chromosome using FISH in patients with TS who did not present Y chromosome when investigated with conventional cytogenetics.

Table 1. Patients karyotype studied with standard cytogenetics and FISH

Patient	Standard cytogenetics			FISH		
	Karyotype	Mosaicism level (2nd line)	Number of analyzed metaphases	Karyotype	Mosaicism level (2nd, 3rd line)	Number of analyzed cells
1	45,X/46,X,i(Xq)	25%	16	45,X/46,XX	12%	250
2	45,X/46,X,+mar	Missing data	48	45,X/46,XX	11%	250
3	45,X	0%	Missing data	45,X	0%	250
4	45,X/46,X,+mar	6%	49	45,X/46,XX	2%	250
5	45,X	0%	Missing data	45,X	0%	250
6	45,X/46,XX	20%	24	45,X/46,XX	23%	250
7	45,X	0%	18	45,X/46,XX/47,XXX	14%	250
8	45,X/46,XX	20%	41	45,X/46,XX	18%	250
9	45X/46X,i(Xq)	Missing data	Missing data	45,X/46,XX	85%	250
10	45,X	0%	100	45,X	0%	250
11	45,X	0%	25	45,X/47,XXX	6.00%	250
12	45,X	0%	9	45,X	0%	250
13	45,X	0%	121	45,X	0%	250
14	45,X	0%	5	45,X	0%	250
15	45,X	0%	7	45,X	0%	250
16	45,X	0%	4	45,X	0%	250
17	45,X/46,X,r(X)	Missing data	Missing data	45,X/46,XX	10%	250
18	45,X/46,X,del(Xp)	Missing data	4	45,X/46,XX	14%	250
19	45,X	0%	84	45,X	0%	250
20	45,X	0%	27	45,X	0%	250
21	45,X	0%	10	45,X	0%	250

Material and method

Twenty-one patients diagnosed with TS by karyotyping were included in the study . Thirteen patients had homogeneous monosomy, 45,X and 8 had a second line cell, of which: 2 - 45,X/46,XX, 2 - 45,X/46,X,i(Xq), 1 - 45,X/46,X,del(Xp), 1 - 45,X/46,X,r(X) and 2 - 45,X/46,X,+mar. The number of metaphases analyzed with standard cytogenetics varied between 4 and 121 (*Table 1*).

Y chromosome material was investigated using FISH. Other sex chromosome abnormalities were also assessed within the same analysis. Each case was evaluated by analyzing 200 interphase and 50 metaphase cells, using centromeric probes for X and Y chromosomes (Vysis®, Abbott). Analyzed cells were peripheral blood lymphocytes. Biologically significant mosaicism cut-off was 2%.

FISH analysis consisted in technical steps to obtain metaphase cells and FISH technique specific stages.

Chromosomes were prepared according to standard techniques for treating lymphocytes from peripheral blood (cell culture using a complete culture medium with PHA, blocking cells in metaphase using colchicine, hypotonic shock using KCl, fixation with acetic acid:methanol in a proportion of 1/3 and spreading on a slide).

Specific stages of FISH technique consisted in: slide pre-treatment (suspension of the slide obtained in successive baths of 1X PBS

Table 2. Literature data regarding the presence of the Y chromosome in TS in conventional cytogenetics

Reference	Number of patients	Analysed metaphases	Patients with Y chromosome (%)
Gravholt et al (12)	114	Not mentioned	6.1
Palmer et al (13)	110	32	5.5
Hanson et al (14)	53	39	11
Vlasak et al (15)	198	Not mentioned	3.5
Mazzanti et al (16)	592	Not mentioned	0
Pelz et al (17)	101	Not mentioned	0
Hall et al (18)	129	Not mentioned	3.9
Ranke et al (19)	150	Not mentioned	0
Jacobs et al (20)	211	100	6.2
Gotzsche et al (21)	179	Not mentioned	2.2
Flynn et al (22)	43	35	2.3
Held et al (23)	87	64	2.3
Park et al (24)	116	Not mentioned	2.6

(5') - PFA (10') – 1 X PBS (5'), suspension in HCl 0.01 N + pepsin at 37° C (8') , suspension in successive bath of 1 X PBS (2') - PFA (10') – 1 X PBS (2'), dehydration in ethyl alcohol 70% (2'), 85% (2'), 100% (2'), successively), hybridization (applying centromeric X, Y probes at 37° C, then at 75° C (5'), placing the slide in a hybridization chamber at 37° C during the night), slide lavage (in a solution of 0,4 X SSC (0.3 M sodium citrate, 3 M NaCl), deposition of the slide on the hybridizer at 70° C (2'), slide suspension in a 2 X SSC solution), DAPI application and examination with a fluorescent microscope. Each patient from the study signed an informed consent.

Results

In this study, FISH did not reveal the presence of the Y chromosome. A centromeric probe for chromosome X confirmed that 17 patients had the same karyotype as in conventional cytogenetics. The two chromosome markers observed in standard cytogenetics were identified by FISH as having the origin in the X chromosome. In patient 11, diagnosed with homogeneous X monosomy by conventional cytogenetics, FISH revealed a second cell line 47,XXX, as a low-level mosaicism (6%) (*Table 1*). Also, in patient 7, a first diagnosis of homogeneous X monosomy became 45,X/46,XX/47,XXX with FISH, the level of mosaicism for the second and third line being of 14% (*Table 1*). In cases with mosaicism identified in standard cytogenetics, the mosaicism level observed by FISH was similar.

Discussion

Using FISH, this study did not reveal the presence of the Y chromosome, but highlighted the importance of this technique in addition to classic cytogenetics to better characterize the karyotype by identifying the origin of the chromosome markers, and also by detecting low level mosaicism involving X chromosome.

Turner syndrome is characterized by a great phenotypic variability and positive diagnosis is yielded by standard cytogenetic examination. This analysis identifies the presence of the Y chromosome in 0-11% of patients with ST, but if only a small proportion of cells contain a Y chromosome, it will not be revealed by this technique anymore (*Table 2*) (12-24). The variation in the proportion of the cases diagnosed with

Table 3. Literature data concerning Y chromosome frequency using FISH in TS patients without evidence of this chromosome in standard cytogenetics

Reference	Number of patients	Patients with Y chromosome (%)	Number of metaphases analyzed by conventional cytogenetics	Number of cells analyzed by FISH
Wiktor et al (8)	46	0	≥30	500
Nazmy et al (27)	27	26	Not mentioned	Not mentioned
Guimaraes et al (28)	21	9,5	Not mentioned	Not mentioned
Fernandez et al (29)	40	2,5	30	2546-1326
Abulhasan et al (30)	20	0	30-50	120-150
Hanson et al (14)	47	4	10-68	161-313
Van Dyke et al (31)	44	0	≥30	500
Reena et al (32)	8	25	Not mentioned	Not mentioned
Present study	21	0	4-121	250

classic cytogenetics may be attributable to the methodology used, especially to the number of cells that have been assessed. Hook's tables show that an analysis of 30 metaphases in standard cytogenetics can detect a mosaicism level superior to 10% with 95% confidence. In order to detect a less than 10% mosaicism it is necessary to analyze a much higher number of cells, which is costly and time consuming.

In our study, patients 7 and 11 have been previously diagnosed with homogeneous monosomy, 45,X, but using FISH technique they showed a second and a third cell line (45,X/46,XX/47,XXX), respectively a second line (45,X/47,XXX). These mosaicisms are unlikely to be detected in standard cytogenetics because 18 metaphases counted in patient 7 or 25 metaphases in patient 11, could not identify a mosaicism level of 14%, respectively 6% (according with Hook's tables) (25). Thus, an advantage of FISH compared to conventional cytogenetic analysis is that it allows the examination of a much larger number of cells (minimum 100) in a short time, allowing the detection of the cryptic mosaicism. Our results are concordant with some studies, which suggest that cryptic mosaicism involving the X chromosome among TS patients may be present in about 10% of patients, while cryptic mosaicism involving the Y chromosome is observed less frequently (3, 9, 26).

Y chromosome detection is often performed by using centromeric probes, but these probes are not useful for the diagnosis of structural abnormalities of the Y chromosome; in these cases complementary probes to sequences located on the entire length of the Y chromosome are recommended.

In literature, in patients with TS in whom the Y chromosome was not detected by conventional cytogenetics, FISH allowed the Y chromosome to be observed in 0 to 26% of patients (*Table 3*) (8,14,27-32). These studies were generally conducted on a small number of patients, and some of them utilized probes that have mapped also on other regions on Y, including SRY, improving in some studies Y chromosome detection (13, 14, 28-30, 32). Studies that used PCR-based techniques have identified a slightly higher percentage of patients who have the Y chromosome, some authors identifying sequences of this chromosome in up to 60% of TS patients; the higher rate of false positives using this technique has to be also taken into consideration (12, 33-36). Although PCR is a sensitive method, it is difficult to quantify the number of cells containing the Y chromosome.

Y chromosome material in patients with TS is often present as small chromosome markers, difficult to be identified with classic techniques

and sometimes unnoticed, especially when considering a small number of metaphases (37). FISH analysis is indicated if a chromosome marker is observed, in order to determine its origin. In our study, in the two patients with chromosome markers, FISH analysis has identified their origin as the X chromosome. These data are concordant with a review of the literature regarding the chromosome markers identified with FISH, wherein it was revealed that from 50 markers observed in patients with TS, 43 were derived from the X, and 7 from the Y chromosome (37).

Early detection of Y-derived material in patients with TS is important because of the risk of developing gonadal tumors; these tumors can be bilateral and can occur from the first decade of life. In this case prophylactic gonadectomy is recommended. Even if the Y chromosome is not encountered in peripheral blood, we cannot rule out the presence of cell lines that contain this chromosome in the gonads; a clinical suspicion in this case could be the virilization (33, 38). Virilization in a patient with TS is considered to have as leading cause the presence of the Y chromosome, therefore virilization is a clue to perform more extensive studies in order to detect this chromosome (1, 38).

It is not known if a routine research of the Y chromosome must be performed in every TS patient in order to prevent gonadoblastoma, but current recommendations are to search for this chromosome only in situations that suggest its presence, such as virilization or chromosome marker visualization.

Conclusion

FISH is useful in TS as an additional technique to standard cytogenetics, as it is sensitive, specific and rapid for mosaicism detection, the identification of chromosome marker origin and Y chromosome evaluation. A precise cytogenetic diagnosis is useful in the clinical management of these patients.

Conflicts of interest disclosure

The authors declare no conflicts of interest.

References

1. Bondy CA. Turner Syndrome Study Group. Care of girls and women with Turner syndrome: a guideline of the Turner Syndrome Study Group. J Clin Endocrinol Metab 2007;92(1):10-25
2. Saenger P, Wikland KA, Conway GS, Davenport M, Gravholt CH, Hintz R, et al. Recommendations for the diagnosis and management of Turner syndrome. J Clin Endocrinol Metab 2001;86:3061–9
3. Medlej R, Lobaccaro J, Berta P, Belon C, Leheup B, Toublanc JE, et al. Screening for Y-derived sex determining gene SRY in 40 patients with Turner syndrome. J Clin Endocrinol Metab 1992;75:1289–92
4. Manuel M, Katayama PK, Jones HWJ. The age of occurrence of gonadal tumors in intersex patients with a Y chromosome. Am J Obstet Gynecol 1976; 124:293–300
5. Verp MS, Simpson JL. Abnormal sexual differentiation and neoplasia. Cancer Genet Cytogenet 1987;25:191–218
6. Looijenga LH, Stoop H, De Leeuw HP, De Gouveia Brazao CA, Gillis AJ, Van Roozendaal KE, et al. POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. Cancer Res 2003;63(9):2244-50
7. Saenger P. Clinical review 48: The current status of diagnosis and therapeutic intervention in Turner's syndrome. J Clin Endocrinol Metab 1993;77(2):297-301
8. Tsuchiya K, Reijo R, Page DC, Disteche CM. Gonadoblastoma: molecular definition of the susceptibility region on the Y chromosome. Am J Hum Genet 1995;57:1400–7
9. Wiktor AE, Van Dyke DL. Detection of low level sex chromosome mosaicism in Ullrich-Turner syndrome patients. Am J Med Genet A 2005;138:259-61
10. Fernández R, Méndez J, Pásaro E. Turner syndrome: a study of chromosomal mosaicism. Hum Genet. 1996;98(1):29-35
11. Nishi MY, Domenice S, Medeiros MA, Mendonca BB, Billerbeck AE. Detection of Y-specific sequences in 122 patients with Turner syndrome: nested PCR is not a reliable method. Am J Med Genet 2002;107:299–305
12. Gravholt CH, Fedder J, Naeraa RW, Müller J. Occurrence of gonadoblastoma in females with Turner syndrome and Y chromosome material: a population study. J. Clin. Endocrinol. Metab 2000;85:3199-202
13. Palmer CG, Reichmann A. Chromosomal and clinical findings in 110 females with Turner syndrome. Hum. Genet 1976;35:35-49
14. Hanson L, Bryman I, Barrenas ML, Janson PO, Wahlström J, Albertsson-Wikland K, et al. Genetic analysis of mosaicism in 53 women with Turner syndrome. Hereditas 2001;134:153-9
15. Vlasak I, Plochl E, Kronberger G, Bergendi E, Rittinger O, Hagemann M, et al. Screening of patients with Turner syndrome for "hidden" Y-mosaicism. Klin Padiatr

1999;21(1):30-4
16. Mazzanti L, Cacciari E. Congenital heart disease in patients with Turner's syndrome. Italian Study Group for Turner Syndrome (ISGTS). J Pediatr 1998;133:688–92
17. Pelz L, Timm D, Eyermann E, Hinkel GK, Kirchner M, Verron G. Body height in Turner's syndrome. Clin Genet 1982;22:62-6
18. Hall JG, Sybert VP, Williamson RA, Fisher NL, Reed SD. Turner's Syndrome. West J Med 1982;137:32-44
19. Ranke MB, Pfluger H, Rosendahl W, Stubbe P, Enders H, Bierich JR, et al. Turner syndrome: spontaneous growth in 150 cases and review of the literature. Eur J Pediatr 1983;141:81-8
20. Jacobs P, Dalton P, James R, Mosse K, Power M, Robinson D, et al. Turner syndrome: a cytogenetic and molecular study. Ann Hum Genet 1997;61:471-83
21. Goltzsche C, Krag-Olsen B, Nielsen J, Sørensen KE, Kristensen BO. Prevalence of cardiovascular malformations and association with karyotypes in Turner's syndrome. Arch Dis Child 1994;71:433-6
22. Flynn MT, Ekstrom L, De Arce M, Costigan C, Hoey HM. Prevalence of renal malformation in Turner syndrome. Pediatr Nephrol 1996;10:498-500
23. Held KR, Kerber S, Kaminsky E, Singh S, Goetz P, Seemanova E, et al. Mosaicism in 45,X Turner syndrome: does survival in early pregnancy depend on the presence of two sex chromosomes? Hum Genet 1992;88:288-94
24. Park E, Bailey JD, Cowell CA. Growth and maturation of patients with Turner's syndrome. Pediatr Res 1983;17:1-7
25. Hook EB. Exclusion of chromosomal mosaicism: tables of 90%, 95% and 99% confidence limits and comments on use. Am J Hum Genet 1977;29:94–7
26. Yorifuji T, Muroi J, Kawai M, Sasaki H, Momoi T, Furusho K. PCR-based detection of mosaicism in Turner syndrome patients. Hum Genet 1997;99:62–5
27. Nazmy NA. Detection of low level sex chromosome mosaicism in Turner syndrome by FISH. Bull Alex Fac Med 2009;45(1):215-20
28. Guimarães J, Almeida Santos T, Barbosa A, Bastos M, Almeida Santos A, Carvalheiro M. Screening for gene SRY by FISH in patients with Turner Syndrome. European Congress of Endocrinology Glasgow, UK, Endocrine Abstracts 2006;11:720
29. Fernandez-Garcia R, Garcia-Doval S, Costoya S, Pasaro E. Analysis of sex chromosome aneuploidy in 41 patients with Turner syndrome: a study of `hidden' mosaicism. Clin Genet 2000;58:201-8
30. Abulhasan SJ, Tayel SM, Al-Awadi SA. Mosaic Turner syndrome: cytogenetics versus FISH. Ann Hum Genet 1999;63:199-206
31. Van Dyke DL, Wiktor AE. Testing for sex chromosome mosaicism in Turner syndrome. Int Congr Ser 2006;1298:9–12
32. Reena Rahayu MZ, Sharifah NA, Zubaidah Z, Ko Ching Huat C, Yusof SM, Idris JM, et al. Identification of Y chromosomal material in Turner syndrome by fluorescence in situ hybridisation (FISH). Med & Health 2008;3(1):22-9
33. Kocova M, Siegel SF, Nalesnick M, Trucco M. Detection of Y-Chromosome Sequences in Gonadal Tissue of Patients with Turner's Syndrome (45,X). N Engl J Med 1994;331:682-3
34. Osipova GR, Karmanov ME, Kozlova SI, Evgrafov OV. PCR detection of Y specific sequences in patients with Ullrich-Turner syndrome: clinical implications, and limitations. Am J Med Genet 1998;76:283–7
35. Lopez M, Canto P, Aguinaga M, Torres L, Cervantes A, Alfaro G, et al. Frequency of Y chromosomal material in Mexican patients with Ullrich-Turner syndrome. Am J Med Genet 1998;76:120–4
36. Coto E, Toral JF, Menendez MJ, Hernando I, Plasencia A, Benavides A, et al. PCR-based study of the presence of Y-chromosome sequences in patients with Ullrich-Turner syndrome. Am J Med Genet 1995;57:393–6
37. Schwartz S, Depinet TW, Leana-Cox J, Isada NB, Karson EM, Park VM, et al. Sex chromosome markers: characterization using fluorescence in situ hybridization and review of the literature. Am J Med Genet 1997;71:1–7
38. Page DC. Y chromosomal sequences in Turner's syndrome and risk of gonadoblastoma or virilisation. Lancet 1994;343:240.