Thai Journal of Obstetrics and Gynaecology
June 2000, Vol. 12, pp. 145-150

CASE REPORT

Identification of Marker Chromosome in a Turner's Syndrome Patient by Molecular and Cytogenetic Analysis

Kitirat Teachatrisak MD, PhD,*
Chongdee Dangrat MSc,*
Manee Ratanachaiyanon MD,*
Surasak Angsuwattana MD,*
Supaporn Vateekul MSc,*
Somchit Ko-anantakul BSc,*
Budsaba Rerkamnuaychoke DMSc.**

*Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital
**Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand 10700

ABSTRACT

Cytogenetic analysis may be unable to identify the marker chromosome of the mos 45,X/46,X,+mar karyotype in a Turner's syndrome (TS) patient. It is reported that approximately half of the unidentifiable markers occurring in TS are Y derivatives. This is a risk factor of developing gonadoblastoma. Thus, it is crucial to know whether a patient has Y- specific sequences. A rapid multiplex PCR-based method to detect X and Y-specific sequences (X-alphoid and SRY sequences) in the same PCR reaction was developed. SRY gene was detected in peripheral blood DNA of a TS patient and confirmed by Fluorescence In Situ Hybridization (FISH). The patient underwent prophylactic laparoscopic gonadectomy.

Key words: Turner's syndrome, Molecular analysis, Marker chromosome, SRY gene, Gonadoblastoma

The chromosome aberrations associated with 40-50% of all Turner syndrome (TS) cases are monosomy X (45,X). Some of the patients have a variety of anomalies of the X chromosome including mosaicism,[1] which is the presence of at least two karyotypically distinct cell types arising from one zygote. The prognosis of a TS patient varies depending on the type of karyotype. The most important considerations are high risk of developing gonadoblastoma or other gonadal tumors, and virilization at puberty if Y or a portion of Y chromosome is present.[2] Individuals at risk for gonadoblastoma are usually advised to undergo prophylactic gonadectomy, or at least gonadal examination via laparoscopy.[3] The percentage of mosaicism with cell line containing a normal or abnormal Y chromosome in TS has been estimated to be 5.5% using cytogenetic analysis which may miss to detect Y-derived marker chromosome.[4] Interestingly, approximately half of the unidentifiable marker chromosomes that occur in TS patients are Y

derivatives.[5,6] Recently, molecular biological studies have contributed to elucidating the unidentifiable marker chromosome by cytogenetic analysis.[7] More sensitive multiplex polymerase chain reaction (PCR) based method to detect Y-specific sequences, SRY gene, have been developed for TS patients.[8,9] Moreover, the combined application of cytogenetic, molecular cytogenetic, Fluorescent In Situ Hybridization (FISH) and PCR technique significantly improved the precision of marker chromosome identification in patients with TS.[10] In this case report we identify the marker chromosome in 45,X/46,X,+mar mosaic patient using PCR of SRY gene and confirm the result using FISH.

Case report

The patient was an 18 year old phenotypically female. She first visited the Gynecologic Endocrinology Clinic at Siriraj Hospital, Mahidol University in early 1999 due to primary amenorrhea and underdeveloped breasts. She frequently had headache and had no sexual activity. Physical examination revealed a good general condition, with the height of 140.5 cm, span of 144 cm, and weight of 38 kg. Gynecologic examination showed Tanner stage I-II of breasts without pubic and axillary hair. Normal clitoris, hymen, vagina with a small size of cervix and unpalpable uterus were identified. Hormonal tests showed a very high serum level of FSH (112.15 mIU/ml) with a slightly elevated serum level of LH (10.82 mIU/ml).

Cytogenetic examination

Karyotype analysis of peripheral blood was performed by standard cytogenetic techniques. Metaphase chromosomes preparing from PHA stimulated peripheral blood lymphocytes were subsequently examined by G-, Q- and C- banding techniques, respectively. At least 50 metaphase cells were examined for each banding technique.

DNA analysis of SRY gene by PCR

Genomic DNA was isolated from 5 ml peripheral blood sample in EDTA including normal male and female controls using standard technique.[11] The concentration of DNA was measured using a spectrophotometer. The SRY gene and centromeric alphoid repeat fragment of X chromosome were amplified using multiplex PCR. The primers used for the detection of SRY gene were the same as the ones described by Berta et al.[12]; Xes7: 5'CCC GAA TTC GAC AAT GCA ATC ATA TGC TTC TGC3' and Xes2: 5'CTG TAG CGG TCC CGT TGC TGC GGT G3'. The PCR product of 609 base pairs was expected from this pair of primer. The primers for X-centromeric alphoid repeat sequences were; X1: 5'AAT CAT CAA ATG GAG ATT TG3' and X2: 5'GTT CAG CTC TGT GAG TGA AA3' which were described by Witt et al.[13] The PCR product of 130 base pairs was expected from this pair of primer. Multiplex PCR amplification was performed with 50-100 ng of genomic DNA, 200 mM of each dNTP, 10 pmol of each primer (X1, X2, Xes2 and Xes7), 1.5 mM $MgCl_2$, 10 mM Tris (pH 8.3), 50 mM KCl, 1.25 u of *Taq* DNA polymerase in a total volume of 25 µl. The mixture was heated to 94°C for 5 min then the reaction was cycled for 20 sec at 94°C, 10 sec at 55°C and 50 sec at 72°C for 35 cycles. The extension step after the last cycle was at 72°C for 7 min. Amplification products were electrophoresed in 2% agarose gel with high molecular weight marker (100 bp ladder DNA) and analysed under a UV transluminator after staining with ethidium bromide. DNA from a 46,XY normal male and a 46,XX normal female were used as controls.

Fluorescent In Situ Hybridization (FISH)

FISH was performed on cultured lymphocytes of this patient to confirm the presence of a Y-bearing cell line. The CEP X spectrum green probe containing alpha satellite DNA sequences which is complementary to the DXZ1 locus at the centromeric region of chromosome X (Xp11.1-q11.1) was used for the identification of X chromosome. Whereas CEP Y spectrum orange probe containing alpha satellite DNA sequences which is complementary to the DYZ3 locus at the centromeric region of chromosome Y (Yp11.1-q11.1) was used to identify Y chromosome. The

hybridization procedure was performed as described by TriGen Kit (AneuVysion, Vysis). Hybridization signals were scored both in metaphase spread and interphase nuclei (100 cells in total).

Results

Cytogenetic examination

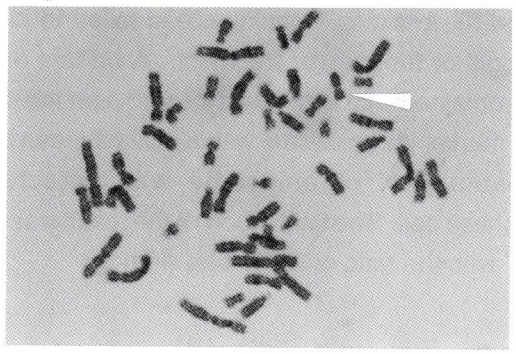
Chromosomal examination of this patient demonstrated mosaicism of the 45,X/46,X,+mar in 50:50 ratio of 100 metaphase cells from G-banding. The marker chromosome looked like chromosome #16 or partial X (Fig 1A). For Q- banding technique, the marker showed a moderately brilliant fluorescent signal at both terminal portions. The signal of one terminal is bigger than the other (Fig 1B). C- banding karyotype also showed the more dense staining at both terminal regions (data not shown). Structural anomalies were not observed in the autosomes. However, the patient's parent chromosomes were not available for investigation.

DNA analysis by PCR of SRY gene

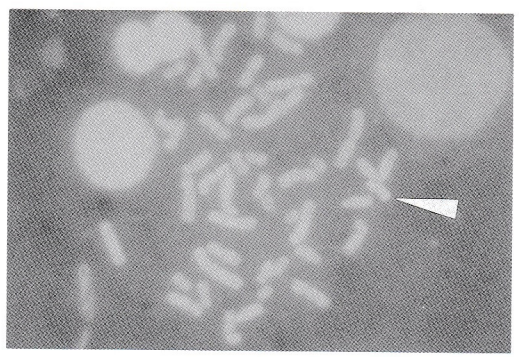
This multiplex amplification showed SRY gene and X-centromeric alphoid repeat sequences in one PCR tube as two distinct bands (609 and 130 bp) in normal male DNA. While the amplification of normal female DNA showed only the 130 bp band. The PCR products of DNA from this patient showed both bands of SRY gene (609 bp) and X- centromeric alphoid repeat sequences (130 bp) (Fig 2). PCR analysis indicated that the peripheral blood DNA of this patient has the region encompassing the SRY gene. Several precautions were taken to avoid false positive results. A female operator, making sample contamination with male cell less likely, performed all reactions, including DNA extraction. Pre- and post- PCR workspaces were strictly separated so that carrying over of amplified DNA sequences to new PCR reactions was prevented.

FISH

Fluorescent in situ hybridization was processed to confirm the presence of Y chromosome material in the marker chromosome which a routine cytogenetic



A.



B.

Fig. 1. Pattern of the marker chromosome from PHA stimulated lymphocyte of the patient. (A), represent G-banding technique (arrowhead). (B), represent Q-banding technique (arrowhead).

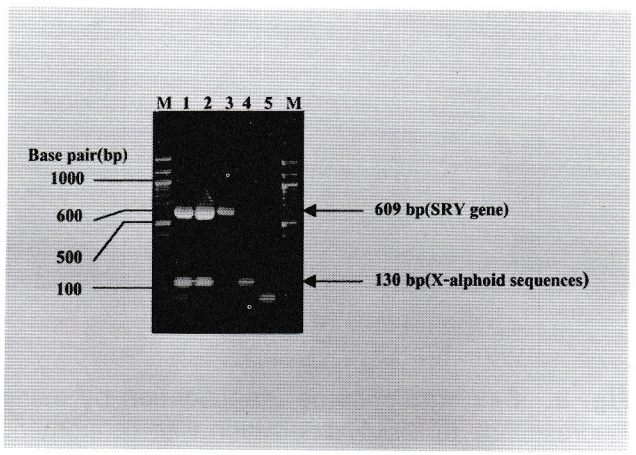
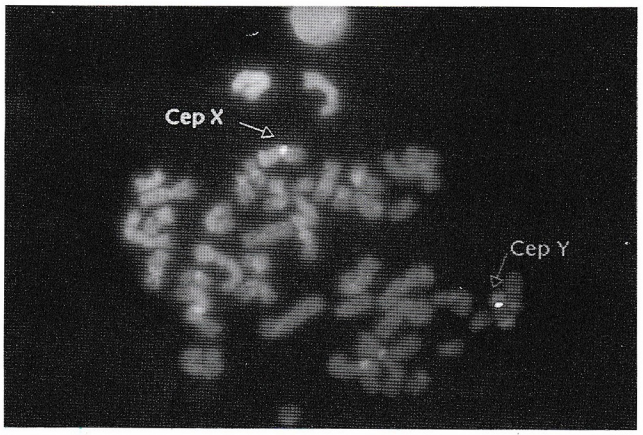
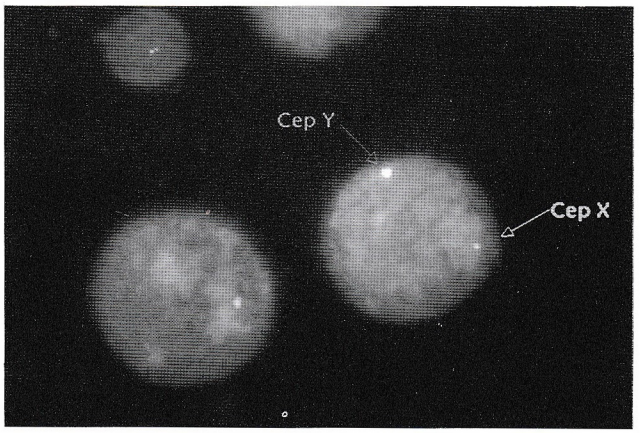


Fig. 2. Agarose gel electrophoresis of the multiplex PCR products using SRY and X-alphoid sequence primers. The 609-bp (arrowhead) and 130 (arrowhead) indicated the present of SRY and X-alphoid products, respectively. Lane M = marker DNA 100 bp ladder, lane 1 = 1st collected patient DNA, lane 2 = 2nd collected patient DNA, lane 3 = normal male DNA, lane 4 = normal female DNA, lane 5 = negative control.

method, G- banding technique, had failed to identify the origin of the marker fragment. A brilliant orange signal obtained with DYZ3 was located near the center of the marker chromosome with a brilliant green signal represented X chromosome was detected in metaphase cell. The final report of the result was 45,X/46,XY with the ratio of 56%: 44% (Fig. 3).



A.



B.

Fig. 3. Sex chromosome identification by using Fluorescence In Situ Hybridization with centromeric probes specific for X and Y chromosomes. CEP Y probe complemented at the centromeric region of Y chromosome. CEP X probe complemented at the centromeric region of X chromosome. (A), represent the signal of CEP Y that indicated on the marker chromosome and CEP X that indicated on normal X chromosome in metaphase cell. (B), represent the signal of CEP Y and CEP X in interphase cell.

Discussion

A marker chromosome is a structurally abnormal chromosome which cannot be identified by conventional cytogenetic analysis, G-banding. Detection of Y mosaicism in TS is a crucial clinical importance because the presence of SRY gene or Y chromosome is a risk factor of developing gonadoblastoma or other gonadal tumor. The relationship between development of gonadoblastoma, dysgenetic gonad and the presence of Y chromosome sequences is well known.[14,15]

Investigation of Y chromosome sequences using PCR has been strongly recommended in all TS patients with evidence of virilization or when a chromosome marker of undetermined origin is found.[16,17] Rapid further investigation to identify the marker chromosome in this patient is the important reason for this multiplex PCR setting. A rapid PCR method for Y chromosomal DNA detection in blood sample has been reported.[12]

PCR of SRY gene, located on Yp11.23,[18] was used to screen Y derived sequences in TS patient. From a previuos report, PCR would provide only one fragment of SRY in a normal control male sample and provide no fragment in a normal control female sample.[19] In this study , we had developed a multiplex PCR reaction for both fragments of SRY gene and X-chromosome in one PCR reaction using SRY fragment (609 bp) as a representing part of Yp chromosome and centromeric region of X-chromosome fragment (130 bp) as an internal control. This reaction was proved to work perfectly for control DNA samples. Thus, this multiplex PCR was applied to the patient's peripheral blood DNA and successfully amplified two positive bands. The result was confirmed by recalling the patient to collect another blood sample for repeating the analysis. Subsequent multiplex PCR amplification again yielded the same results. The marker chromosome in TS patient with mos 45,X/46,X,+mar or 46,X,+mar only was previously reported to be derived from either X or Y chromosome.[5,6,20] In this case, because PCR of SRY gene was positive, marker chromosome was subsequently analyzed by

Q- and C- banding techniques and FISH. Marker chromosome of this patient was an abnormal Y chromosome for the following reasons: 1) it contained SRY gene 2) FISH analysis using CEP Y (Y11.1-q11.1) as a probe showed a single centromere, 3) Q- and C-banding techniques showed heterochromatin signals at both terminals of the marker chromosome. We were not able to identify the exact breakpoint of the abnormal Y chromosome in this patient.

Six months later the patient underwent prophylactic laparoscopic gonadectomy, removal of streak gonads. Histological study showed a focal area of seminiferious tubule in the left streak gonad (∅2x5x15 mm) but no seminiferious tubule in the right streak gonad (∅2x2x15 mm). No sign of malignancy was detected in both gonadal tissues.

In summary, a sophisticated molecular genetic technique, FISH, can eventually disclose the origin of the marker chromosome in this TS patient. However a new rapid, save and sensitive multiplex PCR developed in our laboratory could be utilized for screening the patients at risk before applying the more specific but much more expensive technique as FISH. In fact, PCR is reported to be more sensitive and it can detect Y-chromosome material in as small cell number as 1 of 1000 peripheral blood cells.[8] Thus, this multiplex PCR amplification of SRY gene and X-alphoid sequences is useful for detection of the hidden sequences of Y-chromosome in patients with normal karyotype. This method can also be applied for both prenatal and postnatal diagnosis of fetal sexing.

Acknowledgements

The authors gratefully acknowledge Assist. Prof. Dr. Woranooj Unakul, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, for her critical comments on cytogenetic analysis and Ms. Manusnant Sodhaung for the copy of photography.

References

1. Patsalis PC, Sismani C, Hadjimarco MI, et al. Detection and incidence of cryptic Y chromosome sequence in Turner syndrome patients. Clin Genet 1998; 53: 249-57.
2. Lo KW, Lam SK, Cheung TH, Wong SP, Chang A, Chow JH. Gonadoblastoma in patient with Turner's syndrome. J Obstet Gynaecol 1996; 22: 35-41.
3. Mendes RT, Strufaldi WL, Delcelo R, et al. Y-chromosome identification by PCR and gonadal histopathology in Turner's syndrome without overt Y-mosaicism. Clin Endocrinol 1999; 50: 19-26.
4. Magenis RE, Breg WR, Clark KA, et al. Distribution of sex chromosome complements in 651 patients with Turner's Syndrome. Am J Hum Genet 1980; 32: 79A.
5. Nagafuchi S, Tamura T, Nakahori Y, et al. The majority of the marker chromosome in Japanese patients with stigmata of Turner syndrome are derived from Y chromosomes. Hum Genet 1992; 89: 590-2.
6. Lindgren V, Chen C, Bryke CR, Lichter P, Page DC, Yang-Feeg TL. Cytogenetic and molecular characterization of marker chromosome in patients with mosaic 45,X karyotypes. Hum Genet 1992; 88: 393-98.
7. Larsen T, Gravholt CH, Tillebeck A, et al. Parental origin of the X chromosome mosaicism and screening for hidden Y chromosome in 45, X Turner syndrome ascertained cytogenetically. Clin Genet 1995; 48: 6-11.
8. Osipova GR, Karmanov ME, Kozlova SI and Evgrafov OV. PCR detection of Y-specific sequences in patients with Ullrich-Turner syndrome: clinical implications and limitations. Am J Med Genet 1998; 76: 283-87.
9. Barbosa AS, Ferraz-Costa TE, Semer M, Liberman B and Moreira-Filho CA. XY gonadal dygenesis and gonadoblastoma: a study in two sisters with a cryptic deletion of the Y chromosome involving the SRY gene. Hum Genet 1995; 95: 63-6.
10. Kuznetzova T, Baranov A, Schwed N, et al. Cytogenetic and molecular findings in patients with Turner's syndrome stigmata. J Med Genet 1995; 32: 962-7.
11. Sambrook J, Fritsch EF, Manniatis T. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 1989.
12. Berta P, Hawkin JR, Sinclair AH, et al. Genetic evidence equating SRY and the testis-determining factor. Nature 1990; 348: 448-50.
13. Witt M and Erickson RP. A rapid method for detection of Y chromosome DNA from dried blood specimens by the polymerase chain reaction. Hum Genet 1989; 82: 271-4.
14. Chu CE and Conner JM. Molecular biology of Turner's syndrome. Arch Dis Child 1995; 72: 285-6.
15. Tsuchiya K, Reijo R, Page DC and Disteche CM. Gonadoblastoma: molecular definition of the susceptibility region on the Y chromosome. Am J Hum Genet 1995; 57: 1400-7.
16. Page DC. Y chromosome sequences in Turner syndrome and risk of gonadoblastoma or virilization. Lancet 1994; 343: 240.
17. Kuo PL, Wu RC, Tzeng CC, Lin SJ, Liu SS and Huang KE. Detection of Y-chromosomal DNA with marker chromosomes in Turner syndrome. J Formos Med Assoc. 1995; 94(8): 474-80.
18. Sinclair AM, Berta P, Palmer MS, et al. A gene from the

human sex-determining region encodes a protein with homology to a conserved DNA binding motif. Nature 1990; 346: 240-44.
19. Medlej R, Lobaccaaro JM, Berta P, et al. Screening for Y-derived sex determining gene SRY in 40 patients with Turner syndrome. J Clin Endocrinol Metab 1992; 75: 1289-92.
20. Hook EB and Warburton D. The distribution of chromosome genotypes associated with Turner's syndrome: live birth prevalence rates and evidence for diminished fetal mortality and severity in genotypes associated with structural X abnormalities or mosaicism. Hum Genet 1983; 64: 24-7.