Identification of Sex Chromosome by Fluorescence *in situ* Hybridization

SASAKORN SARASOPHONA, M.Sc.*,
KOTCHAKORN PROMSAWAT, Cert.M.L.T.*,
BUDSABA RERKAMNUAYCHOK, D.M.Sc.*

Abstract

Fluorescence *in situ* hybridization (FISH) is the complicated and very effective technique to determine the origin of chromosome material that cannot be identified by conventional banding techniques. Also determining the hidden sex chromosome and the percentage of mosaicism. Five peripheral blood and one cord blood sample were used to perform centromeric X and Y chromosome-specific DNA probe to determine the sex chromosome. Comparing the percentage of mosaicism between conventional cytogenetic technique and FISH technique, we found a different ratio in mosaicism. That is because the molecular cytogenetic study was the evaluation of chromosome identification in both dividing (metaphase) and non-dividing cells (interphase nuclei).

Key word : FISH, Sex Chromosome

SARASOPHONA S, et al
J Med Assoc Thai 2000; 83 (Suppl. 1): S89-S94

Determining the origin of chromosomal material that cannot be identified by conventional banding techniques, such as marker chromosome or complex karyotype, remains one of the major difficulties of clinical cytogenetics[1]. Classification of the cytogenetically unidentified chromosome is important for establishing phenotype-karyotype correlations[2]. However, in case of mosaicism, over 100 cells would be needed for examination in order to exclude 5 per cent mosaicism with 99 per cent confidence, otherwise loss level mosaicism might be missed[2]. Fluorescence *in situ* hybrization (FISH), is commonly used as an additional diagnostic technique for cytogenetics and it can be per-

* Human Genetics Unit, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand.

formed in both metaphase chromosomes and interphase nuclei. The development of molecular probes by using DNA sequence of differing sizes, complexity, and specificity, coupled with technological enhancements (direct labeling, multicolor probes, computerized signal amplification, and image analysis) make FISH a powerful investigative tool both for basic research and clinical application[3].

We performed dual color FISH with Centromeric X and Y chromosome-specific DNA probes to identify sex chromosomes from five peripheral blood and one cord blood samples in comparison to the conventional cytogenetics.

MATERIAL AND METHOD

Blood specimens

We studied five peripheral and one cord blood samples. In three cases, marker chromosomes were carried out. The fourth case was a male with abnormal phenotype and the other two cases were Klinefelter syndrome.

Karyotyping

Karyotyping analysis was performed by standard cytogenetic technique[4] using metaphase chromosome preparations from PHA stimulated peripheral blood and cord blood lymphocytes. Giemsa bands were obtained by GTG technique[5]. The percentage of cell clones in mosaics, and the replication status of marker chromosomes are presented in Table 1.

DNA Probes

A directly labeled Centromeric X, Y (additional Centromeric 18 probe was used only in case 1) were purchased from Vysis (Downer Grove, IL). The Centromeric X, alphasatellite DNA probe was extended on X p11.1-q11.1 and labeled with Spectrum Green fluorophore. The Centromeric Y, alphasatellite DNA probe was extended on Y p11.1-q 11.1 and labeled with Spectrum Orange fluorophore. The Centromeric 18, alphasatellite DNA probe was extended on 18p11.1-q11.1 and labeled with Spectrum Aqua.

FISH procedure

Slides were denatured in 70 per cent formamide in 2xSSC, pH 7.0, at 72°±1°C for 5 min and immediately dipped in an ethanol series (70%, 85%, and absolute Ethanol) for 3 min. Slides were dried on 45°-50°C slide warmer for 1-2 min. The probe mixture was applied to the glass slide and covered with an 18 x 18 mm coverslip. Hybridization was carried out in a moist chamber at 37°C for a minimum of 6 hours. After hybridization, the slides were washed for 15 min at 42°C in 50 per cent formamide in 2xSSC, pH 7.0 and 2xSSC, respectively. The slides were counterstained with 4', 6-diamidino-2-phenylindole (DAPI : Sigma), mounted with anti-fading solution and covered with coverslips. The slide was observed with an Olympus BX60 fluorescent microscope equiped with a double bandpass filter set for simultaneous observation of Spectrum Green and Spectrum

Table 1. The comparison of cytogenetic results and FISH results.

Case	Age (Year)	Cytogenetics	FISH
1	4 /12	46, XY = 26.7% 47, XY, +mar =73.3%	XY = 100%
2	10 8/12	45, X = 30% 46, X, +mar = 70%	X = 70% 2X = 30%
3	No data	46, X, +mar = 100%	X = 63% 2X = 37%
4	36	46, XY = 95% 47, XXY = 5%	XY = 78.5% XXY = 21.5%
5	6/12	46, XY = 100%	XY = 78.5% XXY = 21.5%
6	40	46, XX = 3.2% 46, XY = 3.2% 47, XXY = 93.6%	XX = 13.43% XY = 30.6% XXY = 55.97%

Orange. Aqua filter was used in case 1 for observation of Centromeric 18. A DAPI filter was applied to find the nuclei. Digital computer imaging was recorded and processed by Cytovision system (Applied Image, UK). The fluorescent signals were counted for 50 metaphases and 150 interphase nuclei.

RESULTS AND DISCUSSION

FISH was undertaken for identification of marker chromosomes in all three cases in whom routine cytogenetic methods had failed to define the origin of small marker fragments. Also, the comparison of mosaicism percentage between conventional cytogenetic result and FISH result in three cases of Klinefelter syndrome was evaluated.

Case 1 with delayed development, minor anomaly, microcephaly, flat nasal bridge, low set ears, hyperpigmental and broad thumbs, had the karyotype of 46, XY/ 47, XY, +marker with a ratio of 26.7 per cent and 73.3 per cent, respectively. We performed FISH by using Centromeric X, Y and 18 specific DNA probes to identify if the marker chromosome had one of these Centromeric probes. Neither of them showed the signal in the marker chromosomes (Fig. 1). Therefore, the marker chromosome did not contain centromeric regions of chromosome X, Y and 18.

Case 2 had a short neck, puffy fraper, broad chest and carrying autle, therefore, the physician requested karyotype analysis. The karyotype showed 45, X/ 46, X, +marker with the ratio of 30 per cent and 70 per cent, respectively. Identification of the marker chromosome by FISH showed the ratio of X/ 2X with 70 per cent and 30 per cent, respectively (Fig. 2). This uncoupling of FISH scoring results and conventional cytogenetics is based on both interphase and metaphase chromosomes. These results have no direct relationship between the degree of mosaicism judged by FISH and conventional cytogenetics has also been reported in uncultured amniotic fluid samples as well as chrorionic villus samples[6,7]. Selective *in vitro* growth may explain the discrepancy. Since the FISH assay is based on uncultured cells, this assay may better reflect the clonal distribution in a sample than conventional cytogenetics based on cultured cells[7].

Case 3, we identified the marker chromosomes of karyotype, 46, X, +marker, with the FISH signal ratio of X/ 2X for 63 per cent and 37 per cent, respectively (Fig. 3). This uncoupled ratio can be explained in the same manner as Case 2.

Case 4 was previously investigated for prenatal diagnosis of amniotic fluid sample and the karyotype showed 46, XY/ 47, XXY with the ratio

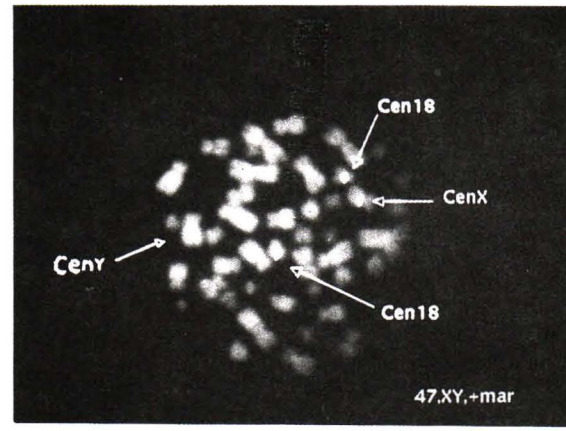


Fig. 1. FISH results using Centromeric X, Y and 18 chromosome-specific DNA probes.
Case 1: Karyotype of 47, XY, +marker. The marker chromosome does not contain Centromeric X, Y or 18. Green is signal specific for Centromeric X, red is for Centromeric Y chromosome and aqua for Centromeric 18.

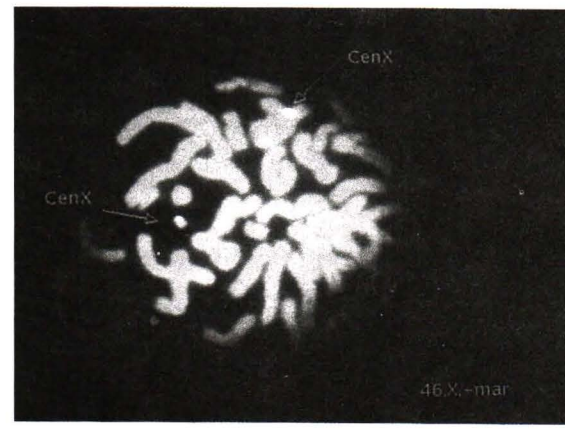


Fig. 2. FISH results using Centromeric X and Y chromosome-specific DNA probes.
Case 2: karyotype of 46, X, +marker. The marker has Centromeric X. Green is signal specific for Centromeric X and red is for Centromeric Y chromosome.

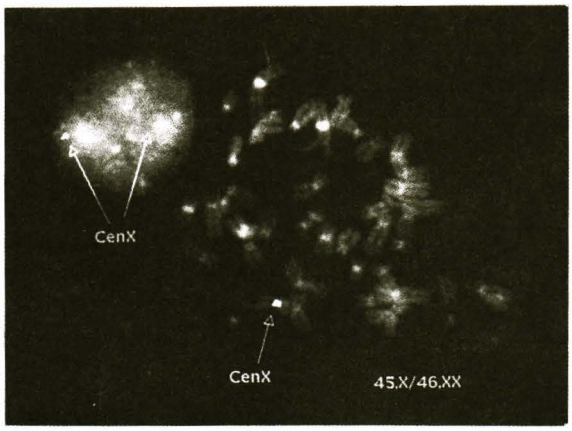


Fig. 3. FISH results using Centromeric X and Y chromosome-specific DNA probes.
Case 3 : karyotype of 45, X/ 46, X, +marker. The marker chromosome has Centromeric X. Green is signal specific for Centromeric X and red is for Centromeric Y chromosome.

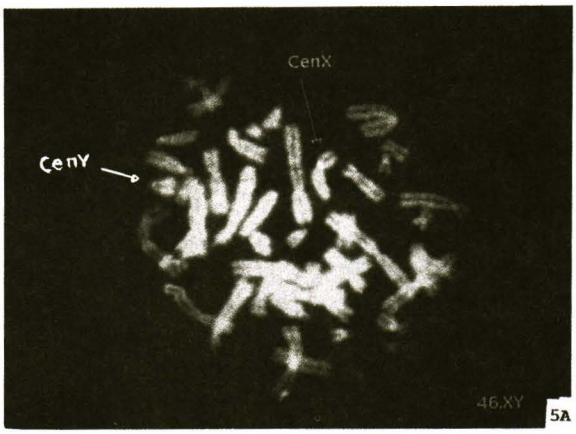


Fig. 5A. FISH results using Centromeric X and Y chromosome-specific DNA probes.
Case 5 : karyotype of 46, XY. Green is signal specific for Centromeric X and red is for Centromeric Y chromosome.

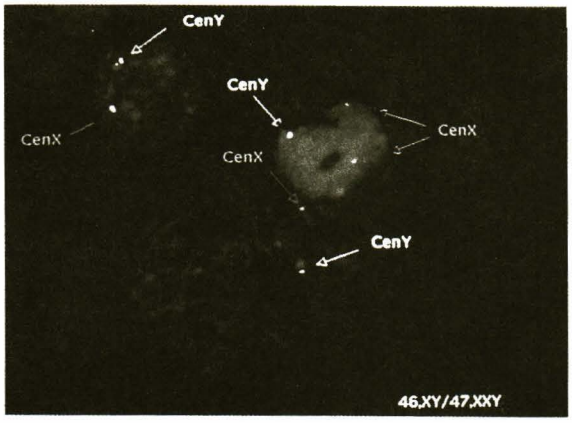


Fig. 4. FISH results using Centromeric X and Y chromosome-specific DNA probes.
Case 4 : karyotype of 46, XY/ 47, XXY. Green is signal specific for Centromeric X and red is for Centromeric Y chromosome.

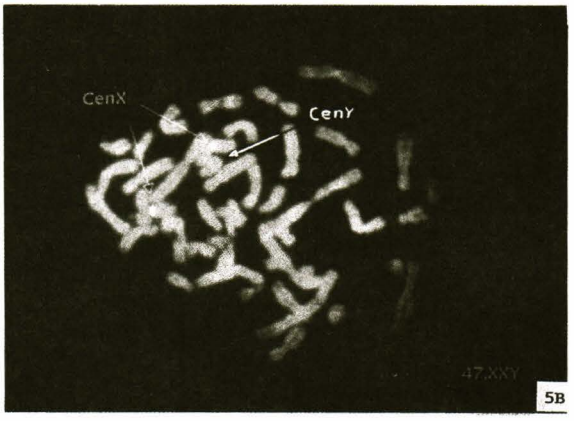
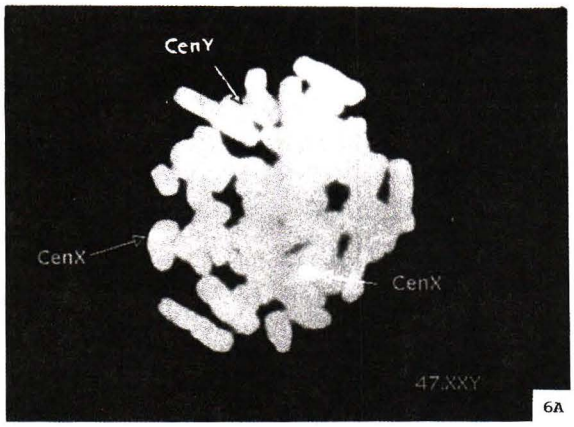


Fig. 5B. FISH results using Centromeric X and Y chromosome-specific DNA probes.
Case 5 : karyotype of 47, XXY. Green is signal specific for Centromeric X and red is for Centromeric Y chromosome.

of 84.4 per cent and 15.6 per cent, respectively. Thus, the result was confirmed by cordocentesis and the karyotype showed 46, XY/ 47, XXY with the ratio of 95 per cent and 5 per cent, respectively, while the FISH results showed the ratio of 78.5 per cent and 21.5 per cent, respectively (Fig. 4).

Case 5, the son of Case 4, at birth had normal karyotype but phenotypic expression of Klinefelter syndrome. He has genetalia, descended testis with cranial growth and development. The FISH results showed XY/ XXY with the ratio of 89 per cent and 11 per cent, respectively (Fig. 5A, 5B).



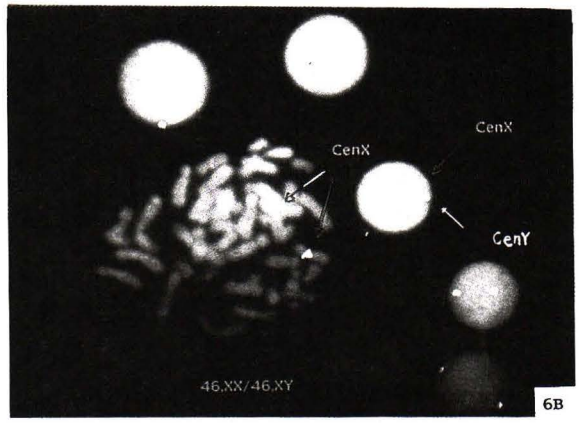


Fig. 6A. FISH results using Centromeric X and Y chromosome-specific DNA probes.
Case 6 : karyotype of 47, XXY. Green is signal specific for Centromeric X and red is for Centromeric Y chromosome.

Fig. 6B. FISH results using Centromeric X and Y chromosome-specific DNA probes.
Case 6 : karyotype of 46, XX/ 46, XY. Green is signal specific for Centromeric X and red is for Centromeric Y chromosome.

Case 6 had 46, XX/ 46, XY/ 47, XXY with a ratio of 3.2 per cent, 3.2 per cent and 93.6 per cent, respectively. He showed the Klinefelter syndrome and Grave's disease with heart block. His FISH results showed XX/ XY/ XXY with the ratio of 13.43 per cent, 30.6 per cent and 55.97 per cent, respectively (Fig. 6A, 6B).

It was reported that the FISH technique has been shown to be more rapid and more reliable than sex chromosome determination by conventional cytogenetic methods or polymerase chain reaction (PCR)[8,9]. Moreover, the advantage of chromosome analysis in both dividing and non-dividing cells would give a better information for counselling than conventional cytogenetics alone.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Sauwakon Ajjimakorn, Dr. Pat Mahachoklertwattana and Miss Orawan Mekmahan for specimen supporting.

(Received for publication on December 1, 1999)

REFERENCES

1. Schwartz S, Depinet TW, Leana-Cox J, et al. Sex chromosome markers : Characterization using Fluorescence *in situ* hybridization and review of the literature. Am J Med Genet 1997; 71: 1-7.
2. Hook EB. Exclusion of chromosomal mosaicism : tables of 90 %, 95 % and 99 % confidence limits and comments on use. Am J Med Genet 1997; 29 : 94-7.
3. American college of medical genetics : Prenatal interphase fluorescence in situ hybridization (FISH) policy statement. Am J Hum Genet 1993; 53 : 526-7.
4. Verma RS, Babu A. Human chromosome : principles and techniques, 2nd edition. New York. McGraw-Hill, Inc. 1995 : 10-1.
5. Verma RS, Babu A. Human chromosome : principles and techniques, 2nd edition. New York. McGraw-Hill, Inc. 1995 : 74-5.
6. Bryndorf T, Christensen B, Vad M, et al. Prenatal detection of chromosome aneuploidies in uncultured chrorionic villus samples by FISH. Am J Hum Genet 1996; 59 : 918-26.
7. Bryndorf T, Christensen B, Vad M, et al. Prenatal detection of chromosome aneuploidies by Fluorescence *in situ* hybridization : experience with 2000 uncultured amniotic fluid samples in a prospective preclinical trail. Prenat Diagn 1997; 17 : 333-41.
8. Harper JC, Delhanty J DA. Detection of chromosomal abnormalities in human preimplantation embryos using FISH. J Assist Repro Genet 1996; 13 : 137-9.
9. Mari C, Shiota K. Sexing of human embryo and fetuses by fluorescent in situ hybridization (FISH) to paraffin-embedded tissues with sex chromosome-specific DNA probe. Am J Med Genet 1994; 50 : 180-6.

การจำแนกโครโมโซมเพศโดยวิธีฟลูออเรสเซ็นซ์ อิน ซิทู ไฮบริไดเซชั่น

ศศกรณ์ สารโสภณ, วท.ม.,*
กชกร พรหมสวัสดิ์, ป.พนักงานวิทยาศาสตร์การแพทย์,*
*บุษบา ฤกษ์อำนวยโชค, D.M.Sc.**

เทคนิคฟลูออเรสเซ็นซ์ อิน ซิทู ไฮบริไดเซชั่น (FISH) เป็นเทคนิคที่ไม่ยุ่งยากในการทำและให้ผลของการตรวจที่แน่นอน และแม่นยำกว่าเทคนิคอื่น ๆ ในการตรวจ marker chromosome, โครโมโซมเพศที่แฝง หรือแม้กระทั่งจำนวนเปอร์เซนต์ไทด์ของ mosaicism ทั้งนี้เนื่องจากการตรวจโดยใช้เทคนิคฟลูออเรสเซ็นซ์ อิน ซิทู ไฮบริไดเซชั่น สามารถตรวจได้ทั้งเซลล์ที่มีการแบ่งตัวให้อยู่ในระยะเมตาเฟสและในระยะอินเตอร์เฟส ในรายงานนี้ทำการศึกษาตัวอย่างเลือด 5 ตัวอย่าง และ cord blood 1 ตัวอย่าง โดยใช้ โพรบที่จำเพาะต่อเซ็นโตรเมียร์ของโครโมโซม X และ Y เพื่อทำการตรวจหาโครโมโซมเพศ และ/หรืออัตราส่วนของโครโมโซมในกรณี mosaicism เปรียบเทียบกับวิธีทางเซลล์พันธุศาสตร์

คำสำคัญ : FISH, โครโมโซมเพศ

ศศกรณ์ สารโสภณ และคณะ
จดหมายเหตุทางแพทย์ ฯ 2543; 83 (Suppl. 1): S89–S94

* หน่วยมนุษย์พันธุศาสตร์, ภาควิชาพยาธิวิทยา, คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี, มหาวิทยาลัยมหิดล, กรุงเทพ ฯ 10400