Two case reports of chromosomal abnormalities using Fluorescence *In Situ* Hybridization (FISH)

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Abstract:

Two case reports of chromosomal abnormalities using Fluorescence *In Situ* Hybridization (FISH)

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We present 2 cases of chromosomal abnormalities. The first patient was 47, XX, +del (18), (pter→q12:). She had delayed development, bilateral club feet and minimal dysmorphic facies. The second case was prenatally diagnosed to paternal derived 46, XY, inv(Y) (p11.2q11.2), t(Y;15) (q12;p12). Physical examination revealed omphalocele, minimal dysmorphic

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facies and bilateral undescended testes. He had normal growth and development at two months. The karyotypes of both cases were verified by using Fluorescence In Situ Hybridization (FISH).

Key words: *FISH, trisomy 18, translocation, t(Y;15)*

บทคัดย่อ:

รายงานผู้ป่วย 2 ราย ซึ่งผลการตรวจโครโมโซมผิดปกติ รายแรกมีโครโมโซม 47 แท่ง โดยแท่งที่เกินมาเป็นโครโมโซมคู่ที่ 18 ซึ่งแขนข้างยาวขาดไปบางส่วน [47, XX,+del(18), (pter→q12:)] ผู้ป่วยมีพัฒนาการช้า เท้าปุก 2 ข้าง หน้าตาวิรูปเล็กน้อย รายที่ 2 เป็นการวินิจฉัยก่อนคลอด ผู้ป่วยมีโครโมโซม 46 แท่ง แต่แขนข้างยาวของโครโมโซมวายเคลื่อนย้าย (translocation) มาติดกับแขนข้างสั้นของโครโมโซมคู่ที่ 15 และมีการกลับส่วน (inversion) ของโครโมโซมวาย [46, XY, inv(Y) (p11.2q11.2), t(Y;15) (q12;p12)] ซึ่งความผิดปกติของโครโมโซมในผู้ป่วยรายที่ 2 ได้รับถ่ายทอดมาจากบิดา ผู้ป่วยมี omphalocele หน้าตาวิรูปเล็กน้อย อัณฑะ 2 ข้างไม่เคลื่อนลงมาในถุงอัณฑะ เมื่ออายุ 2 เดือน การเจริญเติบโตและพัฒนาการอยู่ในเกณฑ์ปกติ ผู้ป่วยทั้ง 2 ราย ได้รับการตรวจยืนยันคาริโอไทป์โดยใช้เทคนิค Fluorescence In Situ Hybridization (FISH)

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Introduction

Chromosomes have been studied using conventional cytogenetics for several years. This method plays a crucial role in diagnosis, prognosis and monitoring of treatment in many fields of medicine. However, some chromosomal abnormalities are difficult to accurately identify.

The Fluorescence *In Situ* Hybridization (FISH) is a combination of molecular technologies and conventional cytogenetics that was first developed in the 1980s.[1, 2] This technique utilizes a fluorescent labeled nucleic acid probe (a single strand fragment of DNA or RNA)[3, 4] hybridized with a complementary target DNA or RNA strand under appropriate conditions. The advantage of FISH is that as an in situ experiment, the DNA is not extracted but studied in its original place, such as on a metaphase chromosome, in interphase nuclei, in a tissue section, or in a blastomere gamete.[1] This technique can be categorized into 2 types: direct and indirect. In the direct method, the fluorescent dye is bound directly to the nucleic acid probe, so that the probe-target hybrid can be visualized under the microscope immediately. With the indirect method, the nucleic acid probe is labeled with a hapten molecule such as biotin, digoxigenin, photobiotin, bromodeoxyuridine or acetylaminofluorence which can be detected by an antibody tagged with fluorescent dye.[1, 3, 4]

FISH has been used in various applications including microdeletion analysis, identification of marker chromosomes, characterization of structural rearrangements, analysis of gene rearrangements associated with neoplasia, ploidy analysis of both prenatal and tumor diagnoses, monitoring of different gender bone marrow transplants, preimplantation analysis, gene amplification studies, and gene mapping.[1, 3, 5] In addition, FISH can also be used in diagnosis of some infectious diseases, especially with difficult or dangerous culture organisms.[3, 5]

This report demonstrates the application of FISH for diagnosis of chromosomal abnormailities that are difficult to determine from routine cytogenetics.

Case reports

Case 1

A 7-year-old girl was the ninth child born to a 45-year-old father and a 42-year-old mother who were not related. There was no family history on either side of congenital abnormalities or mental retardation. The pregnancy was

unremarkable and the patient was born at term by normal delivery. The birth weight was 2,300 g.

Physical examination at 7 years, 2 months of age demonstrated an occipito-frontal circumference (OFC) of 51 cm (50th-97th percentile) and weight of 16 kg (10th percentile). Her dysmorphic features were high forehead, low anterior and posterior hairline, flat supraorbital ridges, diminished lateral eyebrows, pointed chin, upward slanted eyes, short palpebral fissures, bilateral absence of ear lobules, thin upper lip and high arched palate. She also had calf muscle hypoplasia and congenital bilateral clubfeet

A chromosome analysis was subsequently performed, based on the dysmorphic features and developmental delay. Standard lymphocyte culture technique and GTG-banding showed an additional unidentified chromosome (marker chromosome). We suspected that this marker chromosome may have been a deletion from the long arm of chromosome 18. (18 q-, Figure 1A). However, we could not rule out chromosome 21 or Y by GTG banding. QFQ- and NOR-banding were performed and showed that the marker was neither chromosome Y nor 21 (data not shown).

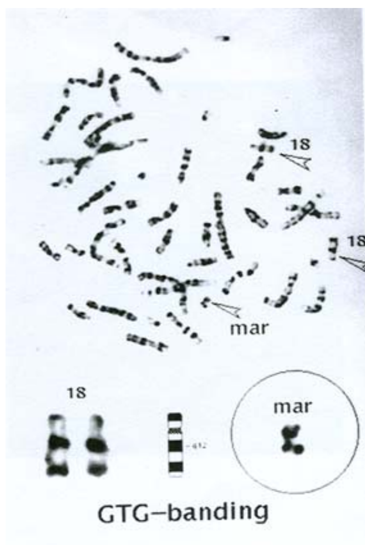

Figure 1A G-banded metaphase chromosomes showing chromosome 18 and a marker chromosome

FISH was performed to give a definite diagnosis using a chromosome 18 centromere probe (cen-18). The result revealed three bright spots from two normal chromosome 18 strands and one from the marker chromosome (Figure 1B). Therefore, the 18q- was identified and the karyotype was designated as 47, XX, +del(18), (pter→q12:).

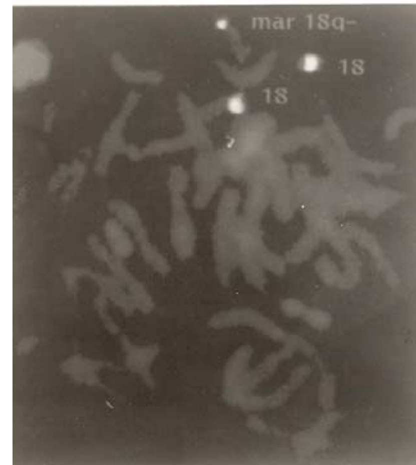

Figure 1B *In Situ* Hybridization of chromosome 18-cen probe to chromosome 18 and marker chromosome (bright spots). This indicated that the marker chromosome was a deletion from the long arm of chromosome 18 (18q-)

Case 2

A 19-year-old woman, gravida 2, para 1, had amniocentesis at 32 weeks gestation determined by last menstrual period (LMP) because of fetal omphalocele. Her husband was 22 years old. They were not related. A 20 ml sample of amniotic fluid was collected with no complications. A routine cytogenetics analysis was performed. Twelve cells from a standard amniotic fluid culture revealed a suspected inversion of chromosome Y (inv Y) and a large short arm on chromosome 15 (15p+, Figure 2A). This could have been a translocation from part of chromosome Y [t(Y;15)]. QFQ-banding was performed to determine if there was chromosome Y material on chromosome 15p+. The result was supportive of our suspicion (data not shown). FISH was subsequently performed using a long arm chromosome Y paint probe (Yqter paint probe) which showed two bright spots on chromosome Y and 15p+ (Figure 2B). Therefore, the Yq;15p reciprocal translocation was identified. The fetal karyotype was desig-

nated as 46, XY, inv(Y) (p11.2q11.2), t(Y;15) (q12;p12). Due to the abnormal fetal karyotype, parental chromosomes were studied to determine if there was an inherited cause. Standard lymphocyte culture and karyotype analysis revealed 46, XY, inv(Y) (p11.2q11.2), t(Y;15) (q12;p12) in the father and normal 46, XX in the mother. The couple decided to continue the pregnancy.

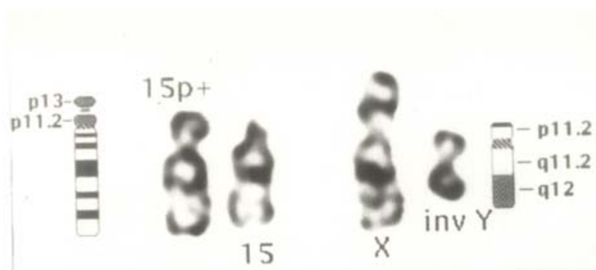

Figure 2A G-banded chromosomes showing chromosome 15p+, 15, X, and Y inversion

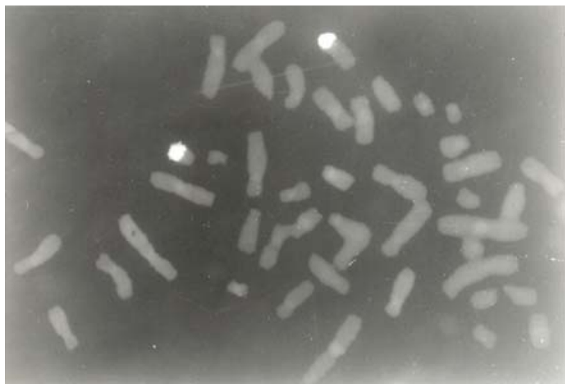

Figure 2B *In Situ* Hybridization of chromosome Yqter paint probe to 15p+ and inv Y (bright spots). This indicated that the 15p+ was chromosome 15 with part of chromosome Y or designated as t(Y;15)

A male infant was born at 34 weeks gestation following premature labor. The birthweight was 2,970 g and the OFC was 33.5 cm (both are normal range for Thai newborns, compared with 36 weeks gestation of Ballard's score), indicating that the gestational age as calculated from the LMP had been wrong. The anomalies detected by examination were bilateral undescended testes and an omphalocele, which was corrected by primary fascial closure during the same admission. The subsequent course after discharge was normal. Physical examination at 2 months of age demonstrated an OFC of 37.5 cm (3rd-50th percentile) and weight of 4,500 g (25th percentile). His face revealed some dysmorphism, described as prominent nose, long philtrum, relative macroglossia and micrognathia. He still had bilateral undescended testes. The surgical scar from the omphalocele correction was healing well, and otherwise the patient showed normal growth and development compatible with his age.

Cytogenetics studies

Lymphocyte cultures were performed in RMPI 1640 (Seromed) with 0.2 ml phytohemagglutinin according to Moorhead, et al.[6] The GTG (G-bands by trypsin using Giemsa) staining technique of Seabright[7] was performed in each case. In addition, QFQ (Q-bands by fluorescence using quinacrine)[8] and NOR (nuclear organizer region) staining[9] were done.

Chromosomes were prepared on slides for FISH by the standard method. FISH was performed according to instructions given with the cytocell probe kit. The slides were dried at 65 °C for 90 min and washed in PBS for 5 min in room temperature, dehydrated through an ethanol series (2 min each at 70%, 85% and 100%) and placed at 37 °C to warm and dry rapidly. A 10 µl of hybridization solution was prewarmed to 37 °C and dropped on the slide. The chromoprobe coverslip was placed on the slide and the edges sealed with rubber glue to prevent evaporation. The slide was placed on a hot plate at 75 °C for 5 min to denature the probe and chromosome, then returned to 37 °C in the dark box overnight with a damp paper towel with 2XSCC. The next day, the coverslip was removed and the slide was washed at 45 °C with 50% formamide/ 2XSCC (for 5 min x 3 times), 2XSCC pH 7.0, 45 °C (for 5 min x 1 time) and PNB buffer at 20-25 °C (for 5 min x 1 time). 10 µl of PI-antifade solution was added for cen-18 and DAPI-antifade solution for Yqter paint probe. Hybridization signals were detected using Cytovision Ultra Applied Imaging.

Discussion

In our first case, FISH was performed to determine the marker chromosome. The cytogenetic analysis of cultured lymphocytes included 47 chromosomes with an additional marker chromosome. The marker chromosome was suspected of having a deletion from the long arm of chromosome 18 (18q-). By using FISH, the 18q- was confirmed. This case was similar to one reported by Wilson et al.,[10] which was not associated with chromosomal translocation. Previous reports found features of trisomy 18 expressed even through there was no trisomy for the short arm of chromosome 18 (18p).[11, 12] Recent molecular studies have demonstrated that the region proximal to band 18q12 does not contribute to the trisomy 18 syndrome. Two important regions, one proximal (18q12.1-q21.2) and one distal (18q22.3-qter), may cooperate to produce the typical trisomy 18 phenotype.[13] Not surprisingly, our case of partial trisomy 18p, and proximal part of 18q12, did not have typical trisomy 18 features. We found that bilateral club feet was the only striking trisomy 18 feature in our patient. This led us to investigate the marker chromosome using a chromosome 18 probe.

The partial trisomy 18 in this case was likely from the maternal age (42 years), which is the most common cause. However, we could not totally rule out an inherited cause. We suggested the parents undergo chromosome studies but they refused, as they did not plan to have another child. We recommended that they use proper contraception until the mother has menopause. If the mother gets pregnant again, we will recommend prenatal diagnosis owing to her age and having the previous child with a chromosomal abnormality.

FISH was used to characterize a structural chromosome rearrangement in our second case. The fetus was prenatally diagnosed as having a reciprocal translocation between chromosomes Y and 15, which is the most frequent translocation involving the long arm of chromosome Y with an autosome.[14, 15] According to Nielsen and Rasmussen[16] the frequency of chromosome Y and autosomal chromosome translocation in the general population is approximately 1 in 2,000. There are two different types of translocation. The common form is when part of Yq is translocated to the short arm of an acrocentric chromosome. Most chromosome Y and acrocentric chromosome translocations involve a break in the heterochromatic part of chromosome Y which does not contain transcribed sequences.[17] Therefore, most commonly t(Y;15) can be found in normal individuals. The second type is when any part of chromosome Y is translocated onto a non-acrocentric chromosome. This often causes an abnormal physical appearance and sterility.[14]

An inversion of the Y chromosome can be found in infertile men[18] or men with undescended testes.[19] Although microdeletion of chromosome Y has been reported in infertile men with undescended testes, no clear correlation has been shown.[20] We could not confirm whether our patient had microdeletion without molecular analysis. In our case, the Y inversion was inherited from his father, so normally he should be fertile like his father. Also, his father had no history of omphalocele nor undescended testes at birth. Therefore, the infant's omphalocele and undescended testes may have been a coincidence. However, as bilateral undescended testes can result in infertility, we will closely follow up the case. If the testes remain undescended, proper treatment must be undertaken. For genetic counseling, we recommended that this family receive prenatal diagnosis in every pregnancy due to the translocation of chromosome Y and 15. If a fetus has chromosome XX (female karyotype) with t(Yq12;15p12), it has no risk of having a male phenotype because the SRY gene is in the Yp11.3 region.

Conclusion

We found that FISH can provide clinical information that is otherwise difficult or impossible to obtain from routine cytogenetics. Our case reports demonstrate one benefit of FISH, in diagnosing chromosome abnormalities.

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