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ORIGINAL ARTICLE

Prenatal exclusion of subtelomeric deletion 1p by fluorescent in situ hybridization

Vorapong Phupong · Verayuth Praphanphoj · Vorasuk Shotelersuk

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Abstract
Background Subtelomeric deletion 1p is difficult to detect from banded karyotypes. Recent developments in the field of molecular cytogenetics have made it possible for submicroscopic rearrangements within chromosomes to be detected using fluorescence in situ hybridization (FISH) techniques.
Materials and methods We describe prenatal FISH testing of subtelomeric 1p deletion in a fetus of a mother whose previous child had subtelomeric 1p deletion.
Results Fluorescent in situ hybridization from fetal cells demonstrated normal 1p, thus predicting a very high likelihood of an unaffected fetus. The study was confirmed by the birth of a normal neonate.
Conclusions We report the use of molecular genetic testing to exclude subtelomeric 1p deletion prenatally. Prenatal diagnostic testing for a known deletion is a reasonable option for couples at risk for having a child with subtelomeric 1p deletion. Molecular testing is more accurate and reliable than ultrasonography and provides families with reassurance.

V. Phupong (✉)
Department of Obstetrics and Gynecology,
Faculty of Medicine, Chulalongkorn University,
Rama IV Road, Pathumwan, Bangkok 10330, Thailand
e-mail: vorapong.p@chula.ac.th

V. Praphanphoj
Center for Medical Genetics Research, Rajanukul Institute,
Dindang, Bangkok, Thailand

V. Shotelersuk
Department of Pediatrics, Faculty of Medicine,
Chulalongkorn University, Rama IV Road, Pathumwan,
Bangkok 10330, Thailand

Keywords Prenatal diagnosis · Exclusion · Subtelomeric deletion · 1p · FISH

Introduction

Terminal deletion of 1p, especially of the segment 1p36.3-1pter, is difficult to detect from banded karyotypes. However, recent developments in the field of molecular cytogenetics have made it possible for submicroscopic rearrangements within chromosomes to be detected using fluorescence in situ hybridization (FISH) techniques which are not detected by conventional chromosomal banding techniques [2]. There have been reports about 1p deletion in patients with multiple congenital anomalies/mental retardation syndromes [3, 6, 7]. Common clinical features that have been suggested as being predictive of a subtelomeric abnormality include developmental delay, mental retardation, prenatal growth deficiency, and a family history of mental retardation [6, 7]. Here, we describe prenatal FISH testing for subtelomeric 1p deletion in a fetus of a mother whose previous child had the deletion.

Materials and methods

Case evaluation

A 31-year-old woman, gravida 2 para 1-0-0-1, went to hospital for a prenatal diagnosis. In 1998, she had the first pregnancy and went to antenatal care at another provincial hospital. The course of her antenatal period was unremarkable. The baby girl was delivered by

cesarean section due to oligohydramnios at 38 weeks' gestation. At the age of 38 days, she began to seize. PE showed many features reminiscent of Prader–Willi syndrome. She had hypotonia, microcephaly, narrow forehead, bilateral epicanthal folds, small almond-shape eyes, abnormal pinnae, small hands and feet, and clinodactyly of fifth fingers bilaterally (Fig. 1). But her clitoris was of normal size. Hearing tests showed left mild and right severe hearing loss. Developmental quotient was 37 at the age of 41 months. Ultrasonogram of kidneys, echocardiogram, and methylation-specific PCR for Prader–Willi syndrome were normal. Conventional cytogenetic analysis demonstrated a normal female karyotype (46, XX). At the age of 6 years, her blood was sent for analysis using a recently developed subtelomeric screening assay, demonstrating a subtelomeric deletion of 1p (Fig. 2a). Both parental blood samples were also obtained and sent for FISH analysis. The results revealed normal study. After knowing the results, the parents were counseled that their risk of having another child with this disease was low since the abnormality appeared to be de novo.

Shortly after, the mother was pregnant and the couple chose to under go a prenatal diagnostic testing for subtelomeric deletion 1p because of a putative germline mutation. Amniocentesis was performed at 16 weeks' gestation without complications. The 20-ml amniotic fluid was sent for both direct cytogenetic analysis and FISH analysis.

The direct cytogenetic preparation showed a male karyotype with no evidence of aneuploidy. FISH was performed and subtelomeric 1p were presented (Fig. 2b). Serial ultrasound examinations at 24, 32, and 38 weeks' gestations revealed normal anatomy and growth of the fetus. At 38 weeks of gestation, the mother was delivered of a normal appearing male infant weighing 4,100 g with Apgar scores 9 and 10 at 1 and 5 min by repeat cesarean section. The baby was still normal at the 1-month follow-up period.

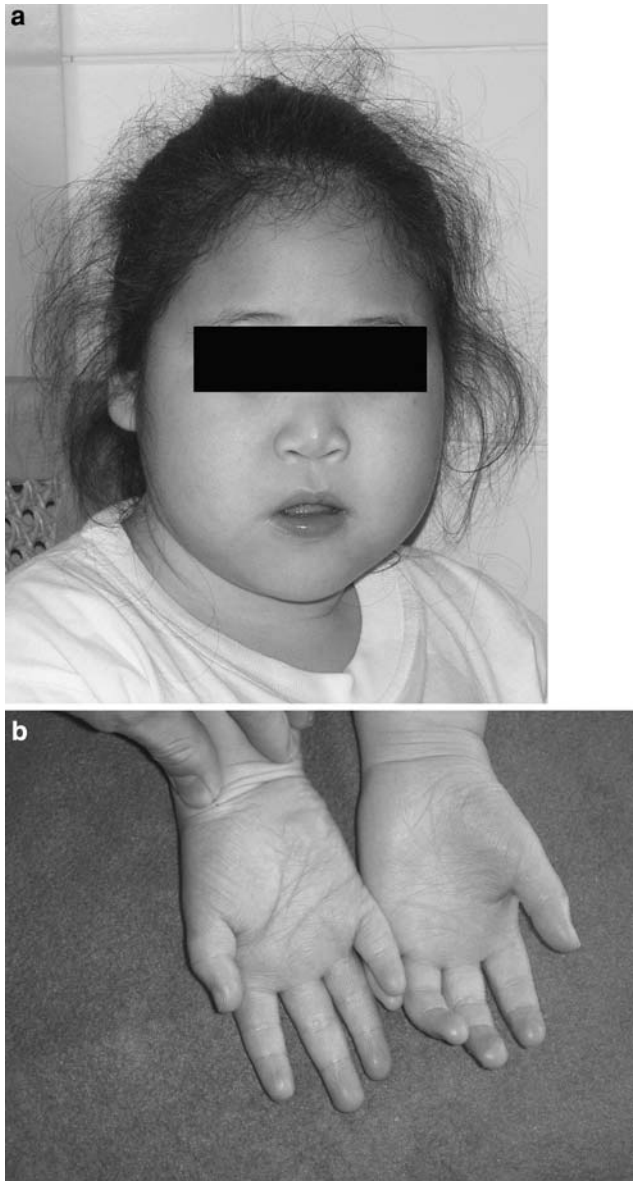

Fig. 1 The first child with subtelomeric 1p deletion. **a** Face. Note obesity, microcephaly, narrow forehead, bilateral epicanthal folds, and small almond-shape eyes. **b** Hands. Note small hands and clinodactyly of fifth fingers bilaterally

FISH analysis and result

Fluorescence in situ hybridization was performed to the metaphase spreads using a set of 41 subtelomeric probes that derived from human BAC/PAC clones. The FISH probes were developed in our laboratory. Some BAC/PAC clones were kind gifts from Dr. David H. Ledbetter while some were purchased from BACPAC. Most clones locate within 1 Mb from the telomeric end of each chromosome. Detail of the clones is available upon request. Dual-color labeling was used to a pair of clones so that each pair of labeled probes will detect both ends of each chromosome simultaneously. When 1p and 1q subtelomeric probes were used (*1ptel, 1qtel*) to the metaphase spreads of the first child, a single hybridization of *1ptel* signal was seen to only one chromosome 1. No additional signal was seen when the rest of the subtelomeric probes were used, confirming a terminal deletion in the counterpart of chromosome 1 where the signal was missing. The findings were consistent in all metaphase spreads examined. Subsequent FISH studies in both parents and the fetal metaphases were normal. For preparing fetal cells, amniocytes were cultured using standard

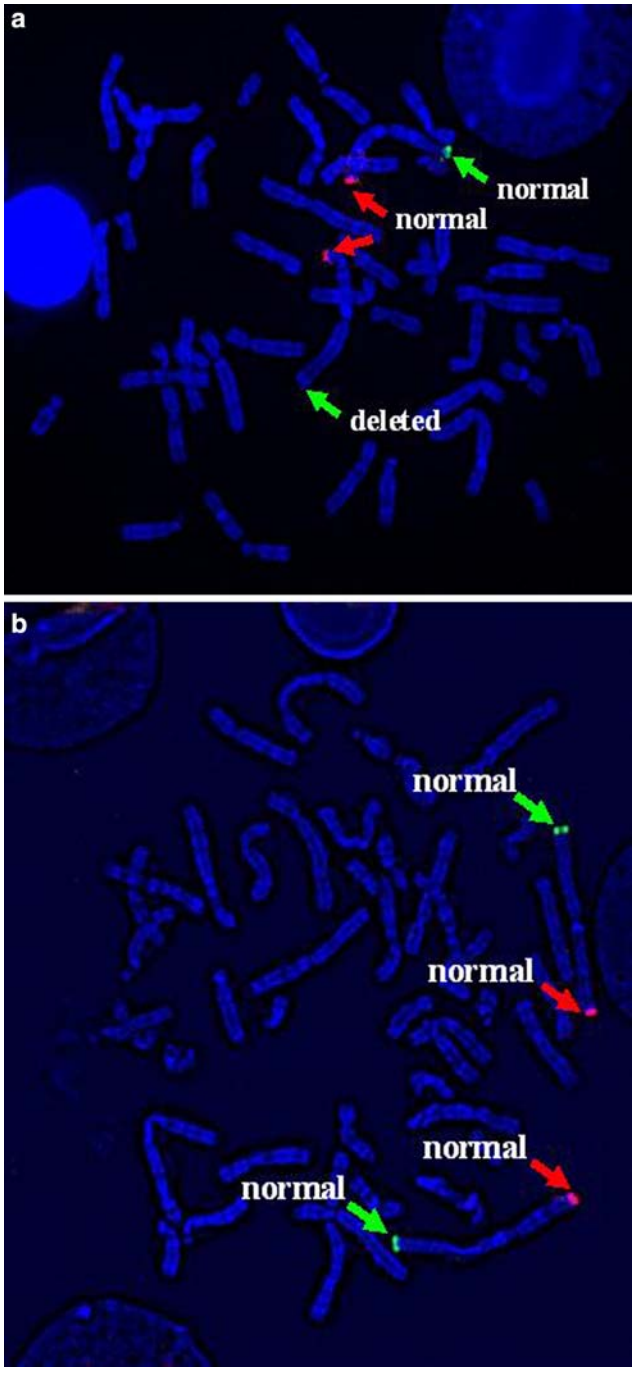

Fig. 2 The FISH result of the affected previous child (**a**) and the unaffected fetus (**b**)

cover slip method in order that each clone would be analyzed separately. Fifteen clones were analyzed by FISH as per standard recommendation. However, in this particular case, we analyzed additional 100 cells from the back-up flask culture to help exclude mosaicism. Thus, the rearrangements appeared to be a de novo event.

Discussion

This case is the first in the literature describing the prenatal exclusion of a subtelomeric 1p deletion by FISH in a second trimester.

Unique sequence DNA for each telomere is located 100–300 kilobases from the chromosome end. Flint et al. [2] have reported that approximately 6% of unexplained mental retardation is accounted for by submicroscopic rearrangement involving subtelomeric DNA. FISH technology using chromosome-specific unique sequence probes has been shown to detect submicroscopic deletions or duplication events [4]. This novel FISH technique requires no additional expensive equipment and, in contrast to alternative methods of multicolor labeling, is relatively uncomplicated.

There have been reports about deletions of chromosome 1p [3, 6, 7]. These reports have led to the delineation of a syndrome consisting of microcephaly, mental retardation, prominent forehead, deep set eyes, depressed nasal bridge, flat midface, relative prognathism, and abnormal ears. The facial features of this first child described here are consistent with those previously described. Additional clinical features present in the patient have also been reported before: seizures in 6 of 8 patients, minor limb abnormalities in 10 out of 12, and skeletal deformities in 5 of 7 [5]. In fact, features of our patient were reminiscent of Prader–Willi syndrome; therefore, we suggest that patients with features of Prader–Willi syndrome but with normal PCR tests should be sent for FISH of subtelomeric 1p.

Severity of the disease, recurrence risks, and risks of complications of prenatal testings were main factors influencing the parents' decisions during the subsequent pregnancy. The reported risks of complications in CVS, early amniocentesis, second trimester amniocentesis and cordocentesis were 3.7, 2.5, 0.5, and 2.7%, respectively [1]. If their next child were affected, the child would be mentally retarded. Therefore, despite a low recurrence risk, they decided to undergo prenatal diagnostic testing for subtelomeric deletion 1p. The couple elected amniocentesis over chorionic villus sampling (CVS) due to the lower risk of fetal loss. Fetal cells were collected for FISH study and the result was negative for the disease.

Alternatively, a couple at a higher risk for having a child with subtelomeric deletion 1p may consider preimplantation genetic diagnosis (PGD). However, PGD requires very sophisticated techniques including single cell FISH and is more expensive.

In conclusion, we report the usefulness of FISH technique in the second trimester to exclude subtelomeric deletion of 1p prenatally. The result of the

prenatal diagnosis allows counseling, and reassuring the families.

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