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폐동맥 협착과 우심실 확장증을 보이는 Pallister-Killian 증후군의 산전진단

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Prenatal Diagnosis of Pallister-Killian Syndrome Associated with Pulmonary Stenosis and Right Ventricular Dilatation

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Pallister-Killian syndrome (PKS) is a rare disorder characterized cytogenetically by tetrasomy 12p for isochromosome of the short arm of chromosome 12. PKS is diagnosed by prenatal genetic analysis through chorionic villous sampling, genetic amniocentesis, and cordocentesis, or by chromosomal analysis of skin fibroblasts, but is not usually detected by chromosomal analysis of peripheral blood cells. Herein, we report a case of a gravida at 23 weeks gestation with pulmonary stenosis and right ventricular dilation of the heart which were detected by sonography. Fluorescence *in situ* hybridization and a multicolor banding technique were performed to verify the diagnosis as 47,XX, +mar.ish i(12)(p10)(TEL++)[16]/46,XX[4], and an autopsy confirmed the cardiac anomalies detected on antenatal sonography. *(Korean J Lab Med 2009;29:366-70)*

Key Words : *Pallister-Killian syndrome, multicolor banding technique, fluorescence in situ hybridization, pulmonary stenosis, right ventricular dilatation*

INTRODUCTION

Pallister-Killian syndrome (PKS), which is also referred to as tetrasomy 12p, was first described by Pallister [1], and later by Killian and Teschler-Nicola [2]. This rare chromosomal anomaly is characterized by tissue-specific mosaicism for a supernumerary isochromosome for the short arm of chromosome 12, i(12p). PKS is diagnosed by prenatal genetic analysis through chorionic villous sampling, genetic amniocentesis, and cordocentesis, or by chromosomal analysis based on clinical characteristics during infancy or adolescence [3-5].

The major ultrasonographic features suggestive of PKS are congenital diaphragmatic hernia, and micromelia, and rare but likely findings include hydrops fetalis, ventriculomegaly in the central nervous system, dilation of the ca-

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vum septum pellicidum, absent visualization of the stomach, and presence of a sacral appendix [6, 7]. Less severely affected individuals may present in postnatal life with mental retardation, epilepsy, skin pigmentation, and facial anomalies, including sparse anterior scalp hair, a flat occiput, hypertelorism, a short nose, a flat nasal bridge, and a short neck [8, 9]. The diverse phenotype of PKS makes the antenatal sonographic diagnosis difficult. There are two reports of PKS in Korea which were diagnosed postnatally by skin fibroblast culture [10, 11]. One report described the prenatal diagnosis of PKS based on nuchal translucency [12]. Herein we report a rare case of PKS diagnosed prenatally that presented with pulmonary stenosis and right ventricular dilation. The diagnosis was confirmed by fluorescence *in situ* hybridization (FISH) and a multicolor banding (mBAND) technique.

CASE REPORT

A 38-yr-old gravida at 22 weeks gestation was transferred to the Department of Maternal Fetal Medicine at St. Paul's Hospital for prenatal genetic diagnosis because of advanced maternal age. Her first child was 15-yr-old with normal growth and development. An ultrasound examination was performed with an ACCUVIX-XQ (Medison Co., Seoul, Korea). On ultrasonography performed at 23 weeks, the biparietal diameter was 5.23 cm (2.2 percentile), the abdominal circumference was 16.47 cm (4.0 percentile), the femur length was 3.19 cm (2.1 percentile), and the estimated body weight was 391 g (1.5 percentile), which was small for the gestational age. The femur length to biparietal diameter ratio was 56%. On cardiac ultrasound, the diameter of the right ventricle and left ventricle was 1.10 and 0.7 cm, respectively, suggesting right ventricular dilatation. In addition, the diameter of the pulmonary artery was much smaller than the aorta, and pulmonary stenosis was suspected. A mild lemon sign was noted in imaging the fetal head and the stomach was clearly observed in a multi-slice view. There were no abnormal findings regarding the amniotic fluid index or abdominal cavity. Following the sonographic examination, an amniocentesis was performed because of the complex nature of the cardiac defects and the advanced maternal age.

Prenatal cytogenetic analysis was performed with the amniotic fluid using an *in situ* culture method according to standard G-banding techniques. The karyotype in the fetus showed 47,XX,+mar in 16 metaphase cells of 12 colonies and 46,XX in 4 metaphases of 3 colonies (Fig. 1). A chromosomal investigation of the parents using peripheral blood samples revealed normal karyotypes for both. We carefully verified the marker chromosome because it was not easy to distinguish the marker chromosome from the long arm of chromosome 21 and another origin [4]. First, FISH analysis using a LSI TEL/AML1 Dual Color, Extra Signal Translocation Probe (Abbott Molecular/Vysis, Des Plaines, IL, USA) was performed to evaluate the origin of the marker chromosome. Two TEL (12p13) signals on both arms of the marker chromosome were noted (Fig. 2). The number of AML1 (21q22) signals was two to rule out an abnormality associated with chromosome 21. Next, the mBAND technique was applied using a chromosome 12 mBAND XCyte probe (MetaSystems, Altlussheim, Germany) to identify the symmetry of both arms of the marker chromosome. The different libraries were combined for each chromosome and labeled with up to five different fluorochromes (DEAC-dUTP, Applied Biosystems; FITC-dUTP, Roche; SpectrumOrange-dUTP, Abott; and Texas Red-dUTP, Molecular Probes; the

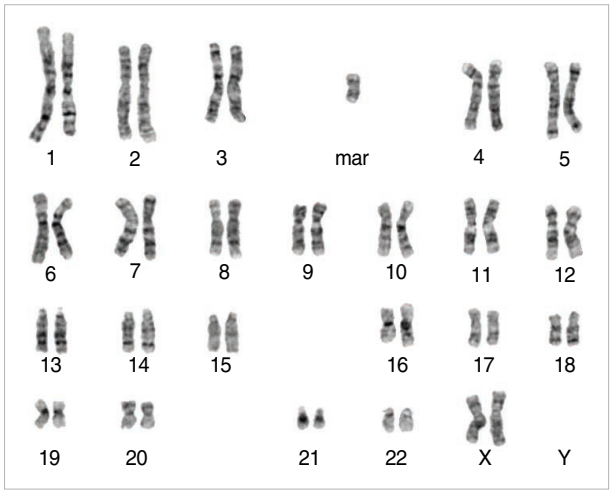

Fig. 1. Conventional cytogenetic analysis on GTG-banded chromosomes from amniotic fluid of the fetus showed 47,XX,+mar in 16 cells and 46,XX in 4 cells.

fifth label was used indirectly via biotin-dUTP, Roche; and detected with StreptavidinCy5, Amersham) [13]. Hybridization, post-hybridization washes, and signal detection of the mBAND kits were carried out following standard protocols [14]. Images were analyzed, captured, and processed under a fluorescence microscope (Axio Imager M1; Carl Zeiss MicroImaging GmbH, Oberkochen, Germany) with the Isis/ mFISH imaging software (MetaSystems). After this, we verified that the marker chromosome consisted of symmetric duplication of the entire short arm of chromosome 12 (Fig. 3).

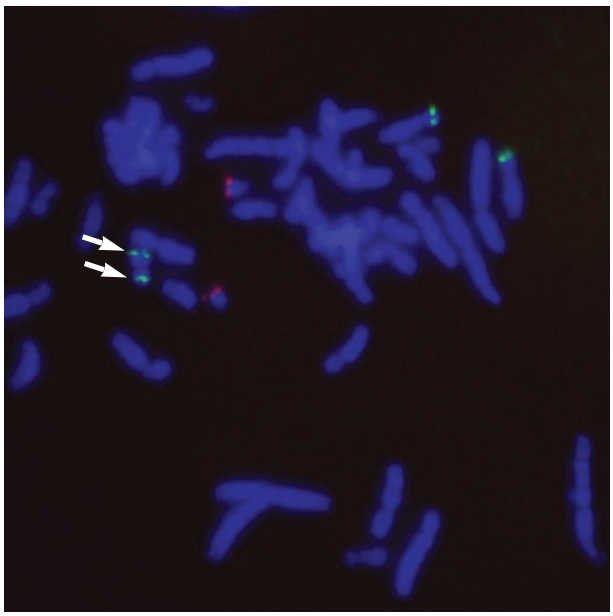

Fig. 2. Fluorescence *in situ* hybridization (FISH) analysis using a TEL/AML1 (LSI TEL/AML1 Dual Color, Extra Signal Fusion Translocation Probe, Abbot Molecular/Vysis, Des Plaines, IL, USA) probe showed two green (TEL) signals located symmetrically on both arms of the marker chromosome (arrows).

The fetal karyotype obtained by GTG banding of amniocytes was confirmed as 47,XX,+mar.ish i(12)(p10)(TEL++)[16]/ 46,XX[4].

Based on the results of fetal chromosomal analysis, genetic counseling was provided and the couple opted for termination of the pregnancy. The postmortem examination revealed dolicocephaly and low set ears. On dissection of the heart, dilatation of right ventricle was noted. The pulmonary artery was much smaller than the aorta and pulmonary stenosis was confirmed.

DISCUSSION

PKS, tetrasomy of the short arm of chromosome 12, shows various forms of tissue-specific mosaicism, but the variation of isochromosome 12p is not correlated with the severity of congenital abnormalities [7, 9]. Maternal age has been considered to be a risk factor of PKS [15]. Advanced maternal age and ultrasound abnormalities are the most common indications for prenatal investigation in reports of PKS, including the present case [7].

To date, there have been approximately 70 reported cases of prenatally-diagnosed PKS, of which only 7 cases had cardiac defects [3, 4, 7, 16-19]. All the cases with cardiac defects, including the present case, are detailed in Table 1. Most of the cases had not only cardiac defects, but other morphologic anomalies. The cardiac defect, however, was the only major abnormal sonographic finding in this case. Fetal growth restriction was another distinct finding from the typical PKS in which fetal macrosomia is common [7].

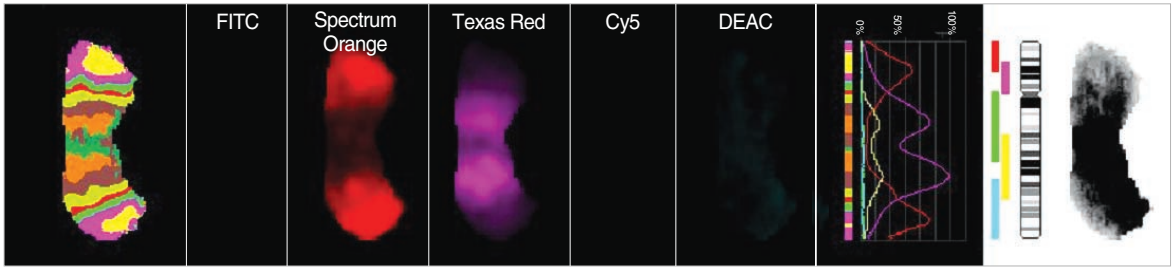

Fig. 3. The chromosome 12-specific mBAND probe kit, XCyte 12 (MetaSystems, Altlussheim, Germany), was hybridized to metaphase cells. Each fluorochrome was detected by adequate filter and the signals were analyzed by Isis/ mFISH imaging software (MetaSystems). This analysis refined chromosome regions involved in isochromosome 12. The multiple band pattern confirmed that i(12) contained all regions of 12p.

Table 1. Prenatally diagnosed Pallister-Killian syndromes presenting with cardiac defects

Reference	Cardiac defects	Extracardiac abnormalities	G-wks	Degree of mosaicism	Molecular cytogenetic method
Gilgenkrantz et al. [4]	Ebstein's anomaly	Polyhydroamnios, rhizomelic micromelia, edema (neck, trunk), facial dysmorphia with macrocephaly*, Club feet*, sacral dimple*	26	100% of amniocytes	-
Wilson et al. [17]	Tetralogy of Fallot	Polyhydroamnios, diaphragmatic hermia, micromelia, facial dysmorphia, pleural effusion, anal stenosis*, rectovaginal fistula*	26	16/18 amniocytes	FISH
Turleau et al. [18]	Right hypertrophy, abnormal tricuspid valve	Polyhydroamnios, micromelia, craniofacial abnormalities*, clinodactyly*, hypoplastic lung, sacrococcygeal pit*	30	100% of amniocytes	-
Lalatta et al. [19]	Atrio-ventricular septal defect	Diaphragmatic hernia, macrocephaly, facial abnormalites*, abscent testicles*	19	0% of 20 clones of amniocytes	-
Langford et al. [3]	Hypoplastic left heart	Increased nuchal translucency, hydrops, diaphragmatic hernia*, micromelia, postaxial polydactyly*, talipes* camptodactyly,* imperforate anus*	15	6% of Chorionic villi	ISH
Doray et al. [7]	Tetralogy of fallot	Dandy walker malformatrion, rhizomelic micromelia, diaphragmatic hernia, club hand, craniofacial dysmorphism*, flexed toes*, various deformity*, adrenal hypertrophy*	16	9/100 Cord blood Lymphocytes	-
Abad et al. [16]	Right ventricular hypertrophy, VSD, severe TR	Increased nuchal translucency, facial abnormalities*	13	100% of amniocytes	
Present case	Right ventricular dilatation, PS	Dolicocephaly, low set ear*	23	16/20 of amniocytes	FISH, mBAND

*Autopsy finding.
Abbreviations: G-wks, gestational week at cytogenetic diagnosis; VSD, ventricular septal defect; TR, tricuspid regurgitation; PS, pulmonary stenosis; ISH, in situ hybridization; mBAND, multicolor banding.

Notably, pulmonary stenosis and right ventricular dilatation have never been documented in the setting of PKS. Although the aneuploid rates of pulmonary stenosis are only 5% [20], our case was proven to have a chromosomal abnormality. It is likely that the presence of another cardiac defect, ventricular dilatation, in our case might, at least in part, be associated with such a chromosomal disorder.

On cytogenetic diagnosis, PKS shows 47 chromosomes and an extra small metacentric chromosome, i(12)(p10). Prenatal diagnosis of PKS is difficult because of the rapid loss of the i(12) in the course of amniocyte subculturing [21]. It is necessary for cultures with slow growth, which require further interpretation of the results during the cytogenetic diagnosis of PKS. In the present case, we used a mBAND technique which is helpful to differentiate the chromosome into region-specific areas at the band level with higher resolution [13]. We confirmed the regions, (12pter→12p10:12p10→12pter), using this method [22, 23].

In conclusion, the present case strengthens the importance of prenatal chromosome analysis for heart defects, particularly for cases with combined heart defects. In cytogenetic analysis, more detailed investigations, such as FISH or mBAND, to complement the standard chromosome analysis may be needed to clarify the nature of the marker chromosome.

요 약

Pallister-Killian 증후군은 tetrasomy 12p 소견을 보이는 드문 염색체 이상으로 주로 isochromosome (등완염색체) 12p가 관찰된다. 이는 산전 융모막 생검, 양수천자, 제대천자술을 통해 진단되며, 출생 후에는 피부 섬유아세포 배양을 통해 진단이 가능하고 혈액 임파구 배양에서는 잘 발견되지 않는다. 저자들은 산전 초음파검사에서 성장지연, 폐동맥 협착, 우심실 확장을 보이는 제태연령 23주 태아에게 시행한 산전 세포유전검사를 통하여 Pallister-Killian 증후군을 진단하였다. 형광제자리부합법과 multicolor banding technique을 시행하여, XX,+mar.ish i(12)(p10)(TEL++)[16]/46,XX[4]를 확진하였으며 부검을 통하여 초음파에

서 발견한 심장기형을 확인하였다.

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