Table 2—Prenatal ultrasonographic findings

Case no.	Cardiac anomaly	Fluid in the fetal stomach	AFV (mm)	Amnioreduction	Cisterna magna (mm)	Other findings
1	Cardiomegaly	+	115	No	26	Single umbilical artery, overlapping fingers
2	SA/SV	+	130	No	25	Single umbilical artery, overlapping fingers
3	VSD	+	74	No	13	Absent
4	NA	+	164	No	20	Overlapping fingers, strawberry-shaped skull
5	HLHS	−	142	Yes	23	Single umbilical artery, overlapping fingers, esophageal atresia, mandibular hypoplasia
6	HLHS	−	107	No	15	Overlapping fingers, strawberry-shaped skull
7	NA	−	140	Yes	12	Overlapping fingers, esophageal atresia, hydronephrosis
8	HLHS	−	149	No	8.5	Single umbilical artery
9	HLHS	−	130	No	20	Overlapping fingers, mandibular hypoplasia, ocular anomalies
10	SA/SV	−	160	Yes	15	Absent
11	Cardiomegaly	−	164	Yes	18	Single umbilical artery, umbilical hernia, esophageal atresia
12	Cardiomegaly	−	114	No	13	Umbilical hernia, clubfeet, radial ray anomalies, rocker-bottom feet, cystic hygroma colli
13	Cardiomegaly	−	96	No	15	Overlapping fingers, diaphragmatic hernia, radial ray anomalies, rocker-bottom feet
14	NA	−	178	Yes	16	Overlapping fingers, diaphragmatic hernia, esophageal atresia, clubfeet
15	VSD	+	152	Yes	NA	Overlapping fingers, hydronephrosis, clubfeet, ocular anomalies, cleft lip
16	Absent	+	135	Yes	6	Single umbilical artery, overlapping fingers, hydronephrosis
17	NA	−	130	Yes	12	Absent
18	VSD	+	69	No	25	Absent
19	PDA, VSD	+	69	No	18	Overlapping fingers
20	VSD	+	NA	No	NA	Absent
21	PDA, ASD, VSD	+	85	No	NA	Absent
22	PDA, ASD	+	106	No	9	Overlapping fingers, rocker-bottom feet
23	Absent	+	80	No	20	Absent
24	VSD	+	126	Yes	5	Single umbilical artery

SA/SV, single atrium/single ventricle; VSD, ventricular septal defect; HLHS, hypoplastic left heart syndrome; PDA, patent ductus arteriosus; ASD, atrial septal defect; AFV, amniotic fluid volume.

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Table 3—Prenatal ultrasonographic and neonatal findings of the 3 groups

	Group 1 ($n = 17$)	Group 2 ($n = 5$)	Group 3 ($n = 2$)
Sex (male)	9 (53%)	0	0
Severe polyhydramnios[a]	12 (70%)	0	1 (50%)
Amnioreduction	8 (66%)	0	1 (50%)
Cardiac anomaly	12 (70%)	5 (100%)	1 (50%)
Stomach not visualized	11 (64%)	0	0
Cisterna magna (mm)[b]	16	17	12
Grade of IUGR	-2.3 ± 0.7	-2.9 ± 0.8	-2.6 ± 0.9
Premature birth	8 (47%)	2 (40%)	0
Cesarean section	1 (5.9%)	4 (80%)	0
Low Apgar score[c]	14 (82%)	2 (40%)	0
NICU	3 (17%)	5 (100%)	0

[a] amniotic fluid pocket >120 mm.
[b] average.
[c] less than 3 after 1 min.
Data are expressed as the number (%) or mean ±SD; IUGR, intrauterine growth restriction.

prognosis. We should probably take into account the severity of cardiac anomalies. Indeed, in our study, 4 cases of hypoplastic left heart syndrome died within 4 days after birth.

Fifteen of the 24 children delivered with trisomy 18 were girls, and no boys survived for longer than 1 month. Previous studies consistently showed more girls born alive and longer survival of girls, for unknown reasons (Root and Carey, 1994).

A recent report suggested that neonatal intensive treatment, including cesarean section, resuscitation by intubation and surgery, improved the survival of neonates affected by trisomy 18: survival rates at ages 1 week, 1 month, and 1 year were 88, 83, and 25%, respectively (Kosho *et al.*, 2006). All cases were prenatally diagnosed, as were those in our study. In our series, the 5 neonates in group 2 underwent active management in the NICU, survived more than 1 month but died within 5 months. It is likely that intensive treatment might improve the relatively short-term prognosis. Future work should further examine the effect as well as the indication of active management on survival for a longer period.

Concerning the mode of delivery, cesarean section was selected for 4 of the 5 neonates in group 2, and transvaginal delivery for the 2 neonates in group 3. These results show that the mode of delivery *per se*, has no impact on the prognosis of trisomy 18; however, cesarean section performed for obstetrical indications such as fetal malpresentation, dystocia, or fetal distress should improve at the least short-term prognosis.

CONCLUSION

Many studies have reported ultrasonographic findings of fetuses affected with trisomy 18; however, the purpose of most studies was to diagnose trisomy 18 in the first or mid-trimester (Shields *et al.*, 1998; Jae *et al.*, 2005; Reinsch, 1997; Ronald *et al.*, 2003). To our knowledge, no study has examined the association between ultrasonographic findings in the prenatal period and neonatal/infant survival possibilities.

We investigated prenatal ultrasonographic findings of pregnant women who delivered live-born infants affected with trisomy 18. Our results showed that survival time less than 1 month was associated with severe polyhydramnios, absence of fluid in the stomach, severe cardiac anomaly and male sex. These findings could be useful for genetic counseling and decision making during pregnancies affected by this condition. The effect of intensive treatment including mechanical ventilation and surgery for cardiac/gastrointestinal malformation is yet to be determined.

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Genital human papilloma virus infection in mentally-institutionalized virgins

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Abstract

Objective. Human papilloma virus (HPV) can cause cervical cancer. Risk factors for HPV infection are primarily related to sexual behavior. We determined the prevalence of HPV infection and abnormal cervical cytology in institutionalized women with no previous sexual experience.

Methods. The study subjects were 251 patients who sought screening for cervical cancer (45.9±9.4 years, mean±S.D., range, 14 to 66). They were institutionalized for psychosomatic disorders since childhood, and had no previous sexual experience. In addition to screening for cervical cancer, specimens for HPV testing were collected.

Results. No women who were positive for HPV DNA was detected, though 251 women without sexual experiences were screened by the hybrid capture 2 test including 26 types of HPV-DNA.

Conclusion. Transmission through means other than sexual intercourse may not exist because we could not detect HPV DNA in 251 women with no previous sexual experience.

Keywords: HPV; Virgins; Cervical cancer screening test; Prevalence of infection

Introduction

The risk factors for HPV infection and cervical cancer are primarily related to sexual behavior, including number of sex partners, life time history of sex partners, and sexual behavior of prior sexual partners [1–4,6–8]. The current guidelines in the U.S. recommend women with normal recent cervical cytology without HPV to be screened every three years. However, how we should manage women with no previous sexual experience, e.g., virgins and physically handicapped women remains unknown, because previous studies have reported that HPV was virtually absent in women with no previous sexual experience [1,3]. So we designed the present study to determine the prevalence of HPV infection and cervical cancer in women with no previous sexual experience. We also discuss the necessity for determining the appropriate duration and frequency of follow-up screening of women with no previous sexual experience.

Materials and methods

The study period spanned from September to December 2006, during which 251 patients who sought screening for cervical cancer were recruited. They had lived in an institution for individuals with psychosomatic disorders (Misakaeno-sono) since childhood, and had no previous sexual experience. They had undergone screening for cervical cancer annually or every 2 years. The results of screening for cervical cancer were normal for the past 12 years.

In this study, 89 conventional cervical cytology and 162 liquid-based cervical cytology (SurePath & CytoRoch, MBL) specimens were interpreted by cytopathologists. Interpretations were rendered by each cytopathologist according to his/her individual application of the diagnostic Bethesda System.

When the subjects underwent screening for cervical cancer, additional specimens for HPV test were collected by using Cytopick (Matsunami Glass Industries, Tokyo). Hybrid Capture 2 kit (Digene Corporation, Tokyo, Japan), which can detect HPV type 6, 11, 16, 18, 30, 31, 33, 34, 35, 39, 42, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 70 and 82, was used for HPV genotyping.

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All study protocols were approved by the Committee for Ethical Issues on Human Genome and Analysis of Nagasaki University.

Results

During the study period, we evaluated 251 females ranging in age from 14 to 66 years (45.9±9.4 years, mean±S.D.). Their admission age were from 2 to 41 years old (15.9±7.8 years old, mean±S.D.). Their institutionalized period were from 2 to 41 years (30.3±8.8 years, mean±S.D.) before the current study. Their types of psychosomatic disorders are shown in Table 1. Virginity was confirmed in all participants by the presence of intact hymen and questionnaire or asking family members.

The results of cervical cytology screening test and HPV DNA test are shown in Table 2. Although 251 women without sexual experiences were screened by the hybrid capture 2 test including 26 types of HPV-DNA, no case of HPV-DNA positive was detected.

One woman was found to have abnormal cervical cytology (Table 2). Since swelling nucleus of parabasal cells were detected but the views suggesting HPV infection were not detected, her result of cytological screening was diagnosed as ASC-US by Bethesda System. She was negative for HPV DNA. She was 56 years old and completely asymptomatic. She was institutionalized for 37 years before the current finding.

Discussion

In the present study, we showed that the prevalence of abnormal cervical cytology was 0.40% (1/251) among females with no previous sexual experience. The result of abnormal cytology detected was ASC-US by Bethesda System.

The low prevalence of abnormal cytology in our cohort with no previous sexual experience compared with females among the general population is probably related to the 12-year negative screening results for cervical cancer. However, we emphasize the necessity for regular follow-up because abnormal cytology was detected in one woman with no previous sexual experience.

Regarding the prevalence of HPV infection, no case of HPV positive was detected among Japanese women with no previous sexual experience surprisingly. Stevens-Simon et al. indicated that HPV is virtually absent in non-abused girls [2]. Furthermore, Gutman et al. suggested that HPV is absent in women with no previous sexual experience [1]. However non-sexual modes of transmission cannot be excluded, such as infection from humid dwellings, contaminated instruments and underwear, and vertical transmission from an infected mother to newborn babies [5].

Table 1
Main disease of the patients

Disease	n=251 (%)
Mental retardation	162 (64.5)
Cerebral palsy	45 (17.9)
Sequela of the encephalitis or meningitis	10 (4.0)
Chromosomal disorder	15 (6.0)
Other diseases	19 (7.6)

Table 2
Results of cervical cytology screening test and HPV DNA test

Bethesda system	n=251 (%)	Number of HPV-positive cases (%)
Negative	250 (99.6)	0
ASC-US	1 (0.4)	0
ASC-H	0 (0)	0
LSIL	0 (0)	0
HSIL	0 (0)	0
SCC	0 (0)	0

Our results suggested that transmission through means other than sexual intercourse may not exist because we could not detect HPV DNA in patients with no previous sexual experience.

For the screening system of cervical cancer in women with no previous sexual experience how we should manage these population remains unknown. To answer this question, we need further follow-up studies to confirm whether the abnormal cervical cytology (the result of screening was ASC-US by Bethesda System) disappears or not in one woman. Up to the present, we did not detect abnormal cytology over 12 years in women with no sexual experience, and we could not detect HPV DNA in patients with no previous sexual experience. The data from this study supports that women with sexual experiences need to be screened longer because of the possibility of chronic infection with high-risk types of HPV. Meanwhile women without sexual experience probably do not need to be screened as often or as long, given the extreme unlikelihood of HPV infection in this patient population.

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Circulating Cell-Free Placental mRNA in the Maternal Plasma as a Predictive Marker for Twin-Twin Transfusion Syndrome

To the Editor:

Twin-twin transfusion syndrome (TTTS), which is a serious complication in monochorionic diamniotic twins (MCDA-T), involves unequal blood flow via the placental vascular anastomoses from the donor to the recipient twin. Although the placental anastomoses are present in all MCDA-T and both fetuses are genetically identical, TTTS occurs in only 15% of MCDA-T, and much of the pathophysiological basis of TTTS remains poorly understood. Clinically, a staging system based on the ultrasound features of TTTS is widely used for the management *(1)* but not for the prediction of TTTS. In addition, the known predictive findings observable by ultrasonographic examination are detectable only in a small portion of TTTS cases *(2)*. New predictive markers are therefore desirable for the early detection and prevention of TTTS. Recently, placental mRNAs, such as human placental lactogen (PL) and some other hormones were detected in maternal plasma, and concentrations of each marker were measured with quantitative real-time reverse transcription (RT)-PCR *(3, 4)*. Thus, circulating cell-free mRNA (cf-mRNA) in maternal plasma has become an attractive target for the noninvasive monitoring of pregnancy disorders *(3, 5)*.

The purpose of the present study was to investigate the use of cf-mRNA concentration in maternal plasma as a predictive marker of later TTTS. The study participants included 17 pregnant women who visited the Obstetrics Clinic of Nagasaki University Hospital at 12–21 weeks of gestation for management of their pregnancy with MCDA-T. Included as a control group were 135 singleton pregnant women without medical complications at similar gestational age. All of the participants gave written informed consent, and the study was approved by the Research Ethics Committee of Nagasaki University. Although none of the 17 cases of MCDA-T were complicated by TTTS at the time of blood sampling, TTTS subsequently developed in 5 cases (TTTS group), but not in the remaining 12 cases (no-TTTS group). Gestational ages at diagnosis of TTTS were 15–25 weeks. The 3 groups had no significant differences in population characteristics, including the maternal age, the number of nulliparous women, and the gestational age at the time of sampling (data not shown).

The blood samples (8 mL) from each woman were collected into an EDTA tube, and the plasma sample was stored at −20 °C until use. After cf-mRNA was extracted from maternal plasma, a quantitative 1-step real-time RT-PCR assay was performed using an ABI 7900T Sequence Detector (Perkin-Elmer) as described previously *(4)*. Primer sets and TaqMan probes for each gene and single-strand, and synthetic DNA oligonucleotides from each amplicon used for a calibration curve were prepared as described previously *(4)*. Then, plasma concentrations of cf-mRNA for human PL and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were measured and converted into multiples of the median (MoM) of the controls adjusted for gestational age, as described previously *(5)*. The differences between the TTTS and the no-TTTS groups were evaluated with the Mann–Whitney *U*-test. Significant difference was defined as a P value <0.05.

The median (minimum–maximum) cf-PL mRNA MoM values were 1.80 (0.89–3.81) in the TTTS-group, 1.14 (0.77–1.35) in the no-TTTS group, and 1.00 (0.82–2.05) in the control group, respectively. At adjusted gestational age the cf-*PL* mRNA concentration was significantly higher in the TTTS group than in the no-TTTS group (Mann–Whitney *U*-test, $P = 0.035$), whereas there was no significant difference of cf-*PL* mRNA concentration between the no-TTTS group and the control group ($P = 0.41$; Fig. 1). In addition, the median cf-GAPDH mRNA MoM value in the maternal plasma was significantly higher in the TTTS

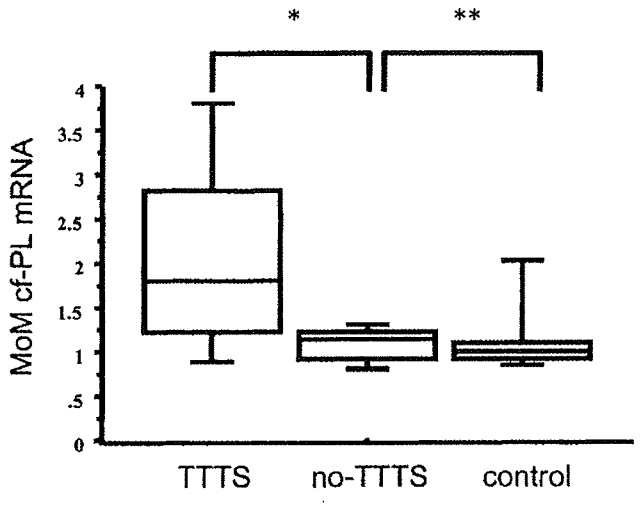

Fig. 1. Box and whiskers plots of cf-PL MoM distribution in the TTTS group, no-TTTS group, and control group.

The median (minimum–maximum) cf-PL mRNA MoM values were 1.80 (0.89–3.81) in the TTTS group, 1.14 (0.77–1.35) in the no-TTTS group, and 1.00 (0.82–2.05) in the control group. * P = 0.035, ** P = 0.41.

group (2.20; range 1.30–2.68) than in the no-TTTS group (1.09; range 0.68–3.25; P = 0.045). Our results suggested the possibility that unapparent pathophysiological changes had already occurred in the women who subsequently developed TTTS, although which specific conditions led to the increased mRNA in the maternal plasma in the TTTS group remain unknown.

In conclusion, a quantitative aberration of both the cf-PL and cf-GAPDH mRNA in maternal circulation may be a novel predictive marker for TTTS, although both statistical differences were small and the sample size was too small to give sufficient strength to the analysis. Therefore, a combination of several cell-free placental mRNA markers could be effective for the prediction of TTTS, similar to the situation for tumor markers. Further study to identify gene transcripts that are expressed only in the placenta and not in blood cells may help to both predict and prevent TTTS and also may further elucidate the pathophysiology of this serious complication.

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SHORT REPORT

A strong association between human earwax-type and apocrine colostrum secretion from the mammary gland

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Abstract Here we provided the first genetic evidence for an association between the degree of apocrine colostrum secretion and human earwax type. Genotyping at the earwax-type locus, rs17822931 within the *ABCC11* gene, revealed that 155 of 225 Japanese women were dry-type and 70 wet-type. Frequency of women without colostrum among dry-type women was significantly higher than that among wet-type women ($P < 0.0002$), and the measurable colostrum volume in dry-type women was significantly smaller than in wet-type women ($P = 0.0341$).

Keywords Human earwax-type · Colostrum secretion · *ABCC11* · Polymorphism

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Short reports

Human earwax, a secretory product of ceruminous apocrine glands, is a dimorphic trait consisting of wet and dry types. We previously showed that a SNP (c. 538G > A, rs17822931) in the *ABCC11* gene is the earwax-type determinant: AA genotype gives dry-type and others wet-type (Yoshiura et al. 2006). As both colostorum and cerumen have a common origin of the secretory glands (Jirka 1968; Petrakis et al. 1975), human earwax type is suggested to be associated with colostrum secretion.

To test this hypothesis, we compared the degree of colostrum secretion to earwax types. The colostrum was obtained from 225 Japanese women on the first postpartum day by 10-min milking both their breasts, and its volume was measured by midwives. Genotyping at rs17822931 was performed as described previously (Yoshiura et al. 2006). The midwives were blind to earwax type of any participants, while obstetricians were also blind to any information on the colostrum secretion status prior to genotyping. All study protocols were approved by IRB, and written informed consent was obtained from all participants. Ninety-two women secreted 0.1–20 ml of colostrum, while the remaining 133 women did not give any recognizable amount of colostrum. Neither the frequency of wet-earwax women without secretion nor the time from delivery to milking differed between 108 primipara and 117 multipara women (data not shown). Genotyping at rs17822931 revealed that 155 of the 225 women were AA homozygotes (dry-type) and 70 GA heterozygotes or GG homozygotes (wet-type). Women in the two groups had no significantly different cesarean-section rates: 33.5% (52/155) of dry-type women versus 38.6% (27/70) of wet-type women ($P = 0.546$, Fisher's exact test). The frequency of dry-type women without colostrum secretion (105/155 or 67.7%)

Table 1 Distribution of dry-type and wet-type women with or without colostrum secreted 24–36 h after delivery

	No. of women examined	No. (%) of women with		*P* value
		No secretion	Colostrum secreted[a]	
All women				
Dry-type	155	105 (67.7)[b]	50 (32.3)[b]	0.000127
Wet-type	70	28 (40.0)	42 (60.0)	
Total	225	133	92	
Primipara				
Dry-type	78	55 (70.5)[b]	23 (29.5)[b]	0.0019
Wet-type	30	11 (36.7)	19 (63.3)	
Total	108	66	42	
Multipara				
Dry-type	77	50 (64.9)[b]	27 (35.6)[b]	0.0297
Wet-type	40	17 (42.5)	23 (57.5)	
Total	117	67	50	

[a] Secretion was defined if the volume was more than 0 ml (ranging from 0.1 to 20 ml)

[b] Fisher's exact test

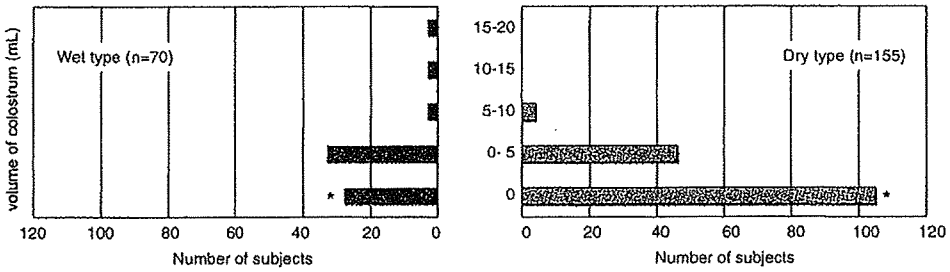

Fig. 1 Distribution of the colostrum secretion volume by earwax type in 70 wet-type women and 155 dry-type women. *Asterisk* represents the frequency of dry-type women without colostrum (105/155) was significantly higher ($P < 0.0002$) than that of wet-type women without colostrum (28/70). Each interval of the colostrum volume includes the right end but not the left end

was significantly higher ($P < 0.0002$, Fisher's exact test) than that of wet-type women without colostrum (28/70 or 40.0%) (Table 1 and Fig. 1). Such a difference was also seen in primipara women without colostrum: 70.5% (55/78) of dry-type women versus 36.7% (11/30) of wet-type women ($P = 0.0019$, Fisher's exact test); and within multipara women without colostrum: 64.9% (50/77) of dry-type women versus 42.5% (17/40) of wet-type women ($P = 0.0297$) (Table 1). Furthermore, the measurable volume of colostrum (average, 1.6 ml) secreted from 50 dry-type women was significantly smaller ($P = 0.0341$, Savage test) than that (average, 4.0 ml) from 42 wet-type women; the 25th, 50th and 75th percentiles of the volume in the dry-type and wet-type women was (0.2, 1.0 and 2.0 ml) and (0.2, 1.1 and 4.0 ml), respectively (Fig. 1).

We have shown that apocrine colostrum secretion from the mammary gland is associated with human earwax-type, leading to an issue that could have very substantial health implications in a wide range of settings from newborn care to cancer etiology. Although several reports suggested a positive association (Petrakis et al. 1975, 1990), our preliminary data denied it (unpublished). Therefore, a role of milk production or lactation initiation in breast cancer remains inconclusive. Endocrine control of lactation develops during pregnancy, and the pituitary gland supplies prolactin and oxytocin as central regulators of apocrine secretion from the mammary gland. Our results suggest that the *ABCC11* gene product (MRP8), an amphipathic anion transporter functioning as an efflux pump (Guo et al. 2003), also plays a role in the colostrum secretion as a peripheral factor independent from the endocrine control. Since there has been no evidence that the colostrum from mothers with dry earwax nourishes their infants less, a role of MRP8 in the colostrum may be confined to its volume. Finally, breast feeding or not, and length of time spent feeding might be associated with colostrum secretion. This could have important implications for anticipatory guidance for mothers planning to breastfeed and based simply on their earwax-type.

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Contraception

Original research article

Effect of placenta previa on blood loss in second-trimester abortion by labor induction using gemeprost

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Abstract

Objectives: The study was conducted to determine whether placenta previa increases bleeding during gemeprost-induced termination of second-trimester pregnancy.

Methods: We carried out a retrospective study of 158 second-trimester terminations between 12 and 21 weeks' gestation. We compared the intraoperative blood loss among three groups: women without placenta previa undergoing gemeprost termination, women with placenta previa undergoing gemeprost termination and women with placenta previa undergoing dilatation and evacuation (D&E).

Results: Eleven of 158 women (7.0%) had placenta previa; four underwent D&E and seven had gemeprost termination. There was no statistical difference in mean intraoperative blood loss among the three groups, although one of the seven women with placenta previa who underwent gemeprost termination developed serious bleeding requiring blood transfusion.

Conclusion: The use of gemeprost for second-trimester pregnancy termination in women with placenta previa seems to be relatively safe and does not increase intraoperative blood loss in the majority of cases.

Keywords: Abortion; Dilatation and evacuation; Gemeprost; Placenta previa; Second trimester

1. Introduction

Placenta previa is one of the most important causes of obstetric hemorrhage [1]. The reported rate of placenta previa at term is approximately 0.5% [2]. Its frequency in the second trimester is significantly higher: approximately 5% at 16 weeks [3,4].

The widespread adoption of fetal screening programs appears to have increased the rate of second-trimester pregnancy terminations in the presence of placenta previa. However, only a few reports have studied the effect of placenta previa on abortion morbidity [5,6]. In most of these reports, dilatation and evacuation (D&E) was used, which is the most common method for second-trimester abortion in the United States [7]. The above reports applied the same procedure to subjects with placenta previa as to those without it, and obtained similar results, that second-trimester pregnancy termination in the presence of placenta previa did not increase bleeding and other forms of maternal morbidity, compared with the outcome of patients without placenta previa.

Prostaglandin analogues are currently the primary alternatives to D&E in labor induction for pregnancy termination in second trimester. Misoprostol is commonly used in the United States [8], while gemeprost is used in Japan. Although uterine perforation occurs far less frequently with labor induction than D&E, labor induction has a greater risk of excessive blood loss [9]. The impact of placenta previa on the technique used for second-trimester termination has been investigated only rarely [10]. In this report, we tested the hypothesis that second-trimester termination by gemeprost is not associated with increased bleeding in patients with placenta previa.

2. Materials and methods

Between January 1994 and January 2006, 178 patients were admitted for termination at 12 to 21 weeks' gestation at Nagasaki University Hospital. The gestational age was

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assessed by the menstrual history and ultrasonographic findings. Pregnancy termination was indicated legally, and written informed consent was obtained from each patient prior to proceeding with treatment.

Just before the termination procedure, all patients were examined by ultrasonography for the presence of placenta previa. Placenta previa was recorded as complete, partial, or low lying. For analysis, we grouped together all the patients with placenta previa. For patients without placenta previa, all abortions were performed after dilating the cervix by laminaria, by the use of gemeprost vaginal pessaries. After removal of laminaria, gemeprost (1 mg) was inserted in the posterior vaginal fornix every 3 h until abortion occurred with a maximum of five gemeprost pessaries over 1 day [9]. On the other hand, for patients with placenta previa, abortions were performed after dilatation of the cervix, either by induction of labor using gemeprost or mechanical evacuation, according to the informed decision of the patient. The uterus was evacuated under general anesthesia using extraction forceps and blunt curettes. Five units of oxytocin or 0.2 mg of methylergometrine maleate was injected intravenously at the completion of the procedure. All patients were treated with prophylactic antibiotics.

We compared the groups with and without placenta previa with regard to patient characteristics. We also compared intraoperative blood loss between patients with and without placenta previa who underwent gemeprost termination, and between patients with placenta previa who underwent gemeprost termination and D&E. Blood loss was quantified by measuring the aspirated amniotic fluid and blood and weighing the gauze used for blood and fluid collection during operation. In our hospital, blood loss is usually recorded in grams because we measure it by weighing the collected fluid in kidney trays or subtracting the weight of dry gauze from that of a blood-soaked one. Since the specific gravity of female blood is approximately 1.053, 1 g is equivalent to 0.95 mL.

Statistical comparison was performed using the two-tailed Student's t test. $p<.05$ was considered significant.

3. Results

We identified 179 patients undergoing second-trimester termination of pregnancy. Of these, 21 were found to be either multifetal pregnancies or lacked documentation about intraoperative blood loss. We analyzed the remaining 158 patients. Table 1 lists the data for patient characteristics and intraoperative blood loss of women with and without placenta previa. Placenta previa was found in 7.0% (11 of 158) of the subjects. There were no significant differences between the two groups with regard to age, parity and gestational age at operation. The mean intraoperative blood loss tended to be larger in the placenta previa group, albeit statistically insignificant (Table 1).

Table 1
Patient characteristics and intraoperative blood loss

	Placenta previa	No placenta previa	p
No. of patients	11 (7.0)	147 (93.0)	NS
Age (years)	28.3±6.8	30.0±6.2	NS
Gravidity	1.3±1.4	1.3±1.4	NS
Parity	0.9±1.1	0.8±2.0	NS
Gestational age (weeks)	16.6±2.7	18.0±2.6	NS
Blood loss (g)	284.1±350	189.6±259	NS

Data are expressed as number (%) or mean±SD. NS, not significant.

Table 2
Characteristics and operative outcome of cases with placenta previa

Case no.	Age	G	P	GA (weeks)	Blood loss (g)	Laminaria	Method
1	31	2	1	17	460	–	Gemeprost
2	27	5	4	19	195	+	Gemeprost
3	21	0	0	14	86	+	Gemeprost
4	41	1	0	18	260	+	Gemeprost
5	17	0	0	16	80	+	Gemeprost
6	28	1	1	19	1272	+	Gemeprost
7	27	1	1	19	60	+	Gemeprost
8	22	1	1	17	330	–	D&E
9	34	0	0	13	142	+	D&E
10	34	1	1	20	140	+	D&E
11	28	2	1	12	100	+	D&E

G, gravida; P, para; GA, gestational age; +, laminaria was used; –, laminaria was not used; method, method used for abortion.

Of the 11 women with placenta previa, labor was induced by gemeprost in seven and D&E in four women (Table 2). The mean intraoperative blood loss for the seven women with placenta previa who underwent termination using gemeprost (344.7 g) was not significantly larger than that of women without placenta previa (189.6 g, $p=.34$) and four women with placenta previa who underwent D&E (178.0 g, $p=.36$).

None of the patients of the two groups bled before, during or after insertion of laminaria. No patient developed uterine rupture, uterine perforation or cervical laceration, and no patient required hysterectomy. Only one woman required blood transfusion because of serious bleeding. She had placenta previa and underwent gemeprost termination (Case 6, Table 2). In this patient, bleeding that developed after the insertion of the first gemeprost pessary was stopped by mechanical evacuation of the uterine content.

4. Discussion

Because it is simple and noninvasive, and does not need any particular skill, labor induction using prostaglandin analogues is becoming increasingly common for second-trimester pregnancy termination. To our knowledge, there is little or no information about the use of this method in the presence of placenta previa. Accordingly, we conducted this retrospective study to determine whether excessive bleeding accompanied second-trimester terminations by gemeprost, the most commonly used agent in Japan, in the presence of placenta previa.

Eleven of 158 (7.0%) women undergoing termination of pregnancy between 12 and 21 weeks had placenta previa.

Seven of the 11 underwent termination using gemeprost. Their mean intraoperative blood loss was 1.8 times greater than that of women without placenta previa, but the difference was statistically insignificant (p=.33).

Blood loss in the seven women who underwent termination by gemeprost was 1.9 times greater than that of four women who underwent D&E, but the difference was also statistically insignificant (Table 2). Although this lack of significance could be due to the small sample size, other reports suggested previously the relative safety of gemeprost for second-trimester termination in women with placenta previa. For example, Thong et al. [9] carried out a retrospective study of 932 second-trimester terminations to determine the efficacy of labor induction by gemeprost. In their study, 80% and 95% of patients aborted within 24 and 48 h, respectively. They did not exclude women with placenta previa from their study group, and overall, only 15 (1.6%) bled more than 500 mL, and 6 (0.6%) required blood transfusion. Significantly, more parous women bled more than 500 mL, but how many of these had placenta previa was not described. After reviewing his experience of 407 second-trimester abortions by labor induction using gemeprost, which comprised approximately 20 placenta previa cases, Deguchi reported that no subjects required laparotomy or blood transfusion because of serious bleeding (K. Deguchi, personal communication, 2005). Considered together, these reports support the notion, although indirectly, that abortion by labor induction using gemeprost during the second trimester is safe and should not be contraindicated even in the presence of placenta previa.

One of seven women in our study developed serious bleeding after the insertion of the first gemeprost pessary, raising the average blood loss of the gemeprost group (Table 2). Although we do not know the details of the case at present, the risk of bleeding should be borne in mind when performing gemeprost termination. That no method other than rapid evacuation of the uterine contents is effective for hemostasis underscores the importance of adequate dilatation of the cervix by laminaria.

Ruano et al. [10] reported that in the presence of placenta previa, second- and third-trimester termination of pregnancy by labor induction carried a substantial risk of hemorrhage. Of the 15 women in their study with complete placenta previa at an average of 22.4 (18–33) weeks' gestation who underwent labor induction including two by gemeprost, four required blood transfusions and one required hysterectomy. They also suggested that feticide before inducing labor might reduce maternal blood loss.

Although our preliminary results lacked statistical power, they show that second-trimester termination by gemeprost was not associated with excessive bleeding in the majority of cases with placenta previa. Intraoperative blood loss in patients with placenta previa varied considerably for unknown reasons. Future work should further examine the effect of gestational age, precise location of the placenta, history of previous cesarean section, presence of placenta accreta or uterine myoma and other factors on blood loss after labor induction by gemeprost for second-trimester termination. Such studies will facilitate identification of women with placenta previa at risk for excessive bleeding in gemeprost-induced second-trimester termination.

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平成２０年度研究成果の刊行物・別冊

American Journal of Medical Genetics Part A 146A:124–127 (2008)

Research Letter

A Girl With Down Syndrome and Partial Trisomy for 21pter-q22.13:

A Clue to Narrow the Down Syndrome Critical Region

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To the Editor:

Down syndrome (DS) is the most common multiple congenital anomaly syndrome with mental retardation. The majority of patients with DS have full trisomy for chromosome 21, but rare patients have partial trisomy 21, indicating the presence of a DS critical region (DSCR) that contains genes contributing to cognitive defects and/or other DS features [Antonarakis et al., 2004]. The DSCR has been narrowed down to a region within 21q22, about 5.4 Mb in length [Delabar et al., 1993; Arron et al., 2006]. Here, we describe a girl with DS and a novel karyotype that may narrow the DSCR down to 2.3 Mb.

The Japanese girl was born with a birthweight of 2,964 g (−0.3 SD), length of 46.8 cm (−1.1 SD) and OFC of 33.0 cm (mean) after 38 weeks gestation to a 22-year-old mother and a 30-year-old father. Diagnosis of DS was made soon after birth. Echocardiogram showed PDA. Although further growth was within the normal range, developmental delay was severe. Physical examinations at age 16 months showed the following abnormalities: brachycephaly with flat occiput, epicanthus, strabismus, upslanting palpebral fissures, small nose with low nasal bridge, upturned nostrils, open mouth, protruding tongue, short neck (Fig. 1a,b), short and broad hands with short fifth fingers, congenital heart defect with heart murmur, joint hyperflexibility and muscular hypotonia. These are 13 of 25 signs and satisfied the criterion for a clinical diagnosis of DS according to the Jackson score [Jackson et al., 1976].

After written informed-consent was obtained from her parents, and the study protocol was approved by the Committee for Genetic Testing and Counseling, Tenshi Hospital, cytogenetic studies were performed. A 550-band-level G-banding analysis on her metaphase chromosomes from cultured peripheral blood lymphocytes revealed an isodicentric chromosome 21, 46,XX,idic(21)(q22) (Fig. 1c). Centromere-dot (Cd) banding revealed that one side of the centromere dots of the isochromosome was separated (data not shown). This finding indicated that one of the centromeres was inactivated; hence, the isochromosome was segregating stably [Maraschio et al., 1980]. Spectral karyotyping (SKY) analysis disclosed a more complex abnormality, that is, 46,XX,t(13;21),idic(21)(q22). This karyotype was confirmed by conventional fluorescence in situ hybridization (FISH) using whole-chromosome-painting probes for chromosomes 13 and 21 (Vysis, Downers Grove, IL; Fig. 1d). Additional FISH analysis

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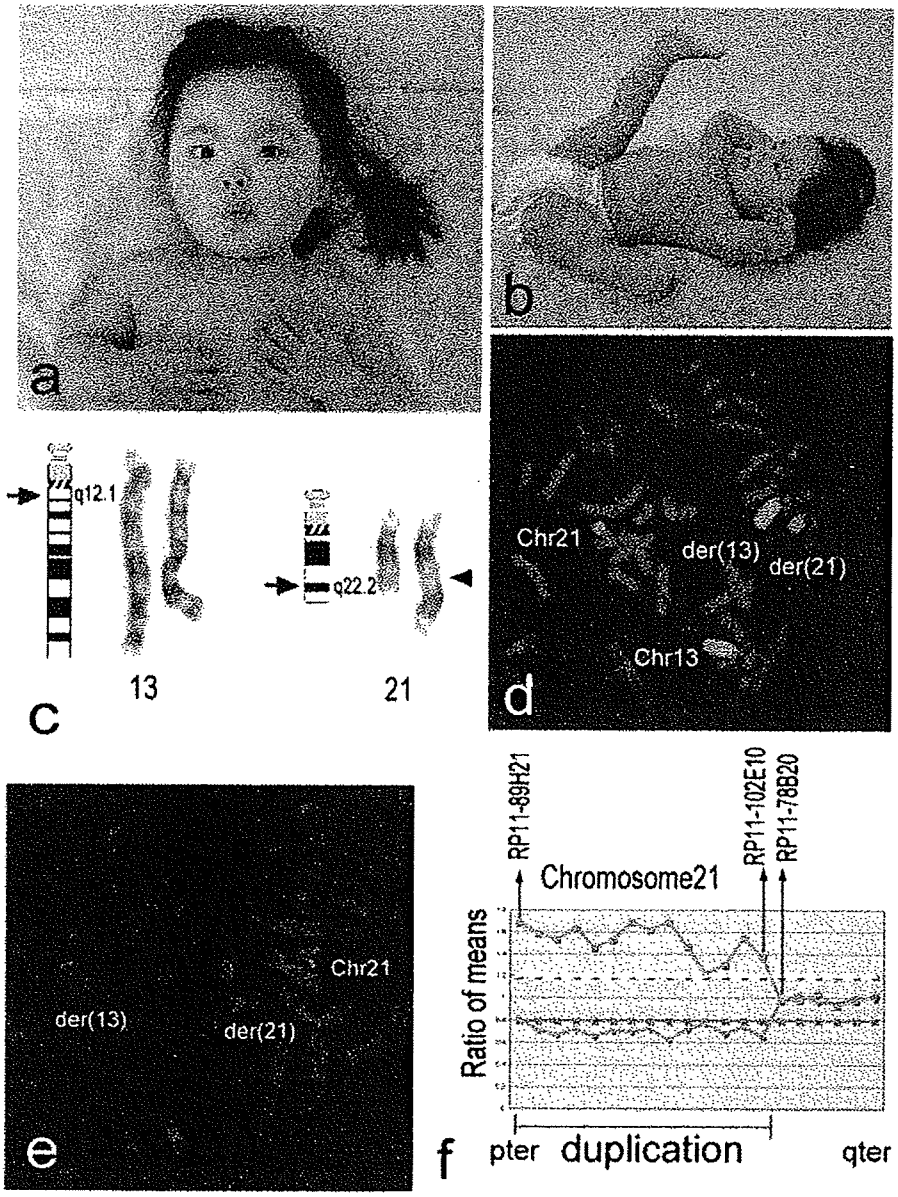

FIG. 1. **a,b**: The patient at age 16 months (a and b). **c**: G-banded partial karyotype of the patient. Arrows indicate the breakpoints of an inserted chromosome 13 and an isodicentric chromosome 21. **d**: FISH with chromosome-specific painting probes for chromosomes 13 (red) and 21 (green), showing an insertion of a chromosome 21-derived segment into a pericentromeric region of 13q. **e**: FISH using BAC-clone probes, RP11-1012D8 (green) and RP11-124E9 (red). Green signals appear on both der(13) and der(21) as well as normal chromosome 21, while red signals are seen on normal chromosome 21 and der(13), but not observed on der(21). **f**: Array CGH analysis demonstrates a duplication of a part of chromosome 21. The proximal and distal end clones within the duplication region were RP11-89H21 (21q11.2) and RP11-102E10 (21q22.13), respectively. The breakpoint of der (21) was suggested to be located between RP11-102E10 and RP11-78B20.

with a BAC clone (RP11-1012D8) located to 21q22.13 showed split signals both on der(13) and der(21) chromosomes as well as on normal chromosome 21. FISH using RP11-89H21 (21q11.2), RP11-166F15 (21q22.13), RP11-98O13 (21q22.13), RP11-183O20 (21q22.13), and RP11-95G19 (21q22.13) all gave signals only on both der(21) and normal chromosome 21, while signals appeared on both der(13) and normal chromosome 21 when using other BAC probes located more distantly, such as RP11-608F9 (21q22.13), RP11-749M19 (21q22.2), RP11-814F13 (21q22.3), RP11-124E9 (21q22.3), and RP11-135B17 (subtelomeric region). These analyses successfully identified the breakpoint of the isochromosome at band 21q22.13 (the UCSC genome browser, 2004 May version, http://genome.ucsc.edu/cgi-bin/hgGateway). As for the derivative chromosome 13, we could not define an insertion point precisely, because the insertion occurred closely to the centromeric region where no BAC probes were available. Within the insertion, a FISH signal for RP11-124E9 (SpectrumOrange) mapped at 21q22.3 was observed

proximally to that for RP11-1012D8 (Spectrum-Green) at 21q22.13 (Fig. 1e), demonstrating an inverted insertion on chromosome 13. All these findings indicated that the patient had partial trisomy for a 21pter-q22.13 segment, while her 21q22.13-qter region was disomic. The distal boundary of the trisomic segment lies at the region corresponding to the BAC clone, RP11-1012D8. As her parental karyotypes were normal in both Q-banding and SKY analyses, the patient's karyotype was interpreted as 46,XX,psu idic(21)(q22.13) ins(13;21)(q12.1;q22.13q22.3)dn (Fig. 2a). To know the presence of any other chromosomal aberrations, home-made whole-genome BAC-based microarray with 1.5 Mb resolution comparative genomic hybridization (array CGH) [Sato et al., 2007] was carried out using the patient's DNA. Consequently, the array CGH detected the same partial trisomy 21 (Fig. 1f), but no other deletion nor amplification in the genome.

The DSCR has always been somewhat controversial since many reports included were either premature for precise chromosome banding, include mosaic individuals, or resulted from familial translocations which may have involved undefined reciprocal deletions [Ronan et al., 2007]. Olson et al. [2004] reported that trisomy for the DSCR alone is insufficient and largely unnecessary to cause specific DS phenotypes in mice models. But, the suggestion may not be suitable for human because there are some differences between mouse and human gene content. Later, Olson et al. [2007] reported that trisomy for the DSCR is necessary for brain phenotypes of trisomic mice, thus the DSCR must have some association with the occurrence of DS. Furthermore, recent experiments using mouse models of DS suggested that a 1.5-fold increase in dosage of *DSCR1* and *DYRK1A* within the DSCR destabilizes a regulatory circuit in a cooperative way, contributing to the reduced NFATc activity and many of the characteristics of DS [Arron et al., 2006].

Interestingly, the trisomic segment of our patient partially overlaps the previously estimated DSCR at 21q22 and we have also confirmed that no known genes were disrupted at the breakpoint (Fig. 2b). With the knowledge gained from certain papers of accurately identified partial trisomy 21 that have been published in the past few years [Forster-Gibson et al., 2001; Rost et al., 2004; Kosaki et al., 2005; Kondo et al., 2006; Ronan et al., 2007], we narrow down the DSCR to a region between the proximal boundary of DS1 [Ronan et al., 2007] and the distal

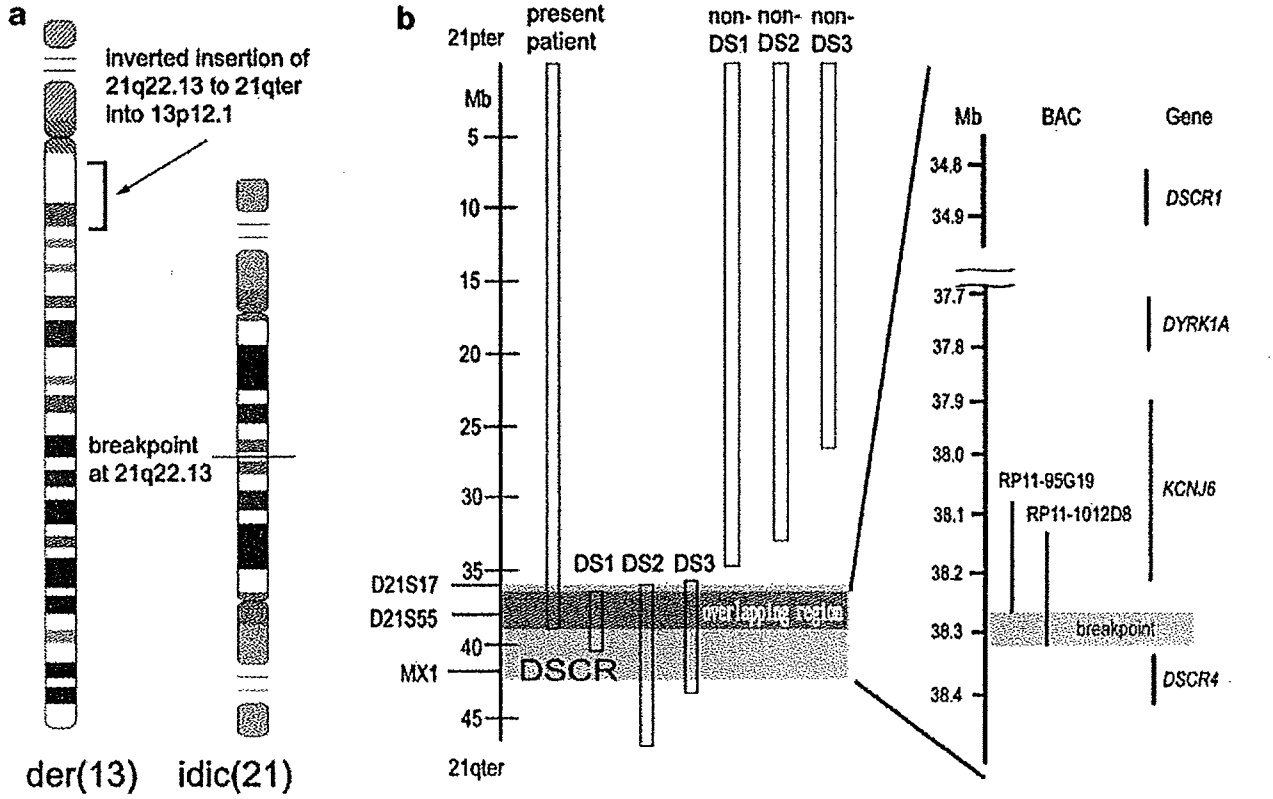

Fig. 2. a: Ideogram of der(13) and der(21) of the patient, showing trisomy for 21pter-q22.13 and disomy for 21q22.13-qter. b: Relationship between the DSCR and trisomic segments of the present DS patient and recently reported three non-DS cases; non-DS1 and DS2 [Kondo et al., 2006] and non-DS3 [Rost et al., 2004] and three other DS patients; DS1 [Ronan et al., 2007], DS2 [Forster-Gibson et al., 2001], and DS3 [Kosaki et al., 2005]. Enlargement of the overlapping region containing RP11-1012D8 with split FISH signals and its contiguous clone (RP11-95G19) without split signal. *KCNJ6* and *DSCR4* are the closest known genes flanking the breakpoint.

border of our patient, from RP11-957K9 located on 21q22.12 to RP11-1012D8 on 21q22.13, approximately 2.3 Mb in size (Fig. 2b) from the previous estimated 5.4 Mb DSCR at 21q22. [Delabar et al., 1993; Arron et al., 2006]. Since our patient manifested most of the main features of DS, we propose that the newly limited DSCR plays a role of the occurrence of DS in humans. A segment distal to the RP11-1012D8 may not attribute to the DS phenotype as far as our patient is concerned. Our data may support the studies by Arron et al. in mice. Because *DYRK1A* gene is included in the DSCR described here but *DSCR1* is not (Fig. 2b), key role of *DYRK1A* could be applied for human.

In conclusion, we have reported on a girl with DS who had a de novo 46,XX,psu idic(21)(q22.13) ins(13;21)(q12.1;q22.13q22.3) karyotype that might provide a potential clue to minimize the DSCR.

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Research Letter

Pre- and Postnatal Overgrowth in a Patient With Proximal 4p Deletion

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To the Editor:

Terminal and interstitial deletions encompassing 4p16 result in Wolf–Hirschhorn syndrome (WHS) and the Pitt-Rogers-Danks syndrome (PRDS) [Wright et al., 1998]. A more proximal, interstitial deletion involving p16.1-p14 shows a distinct clinical entity without overlapping features with WHS and/or PRDS, and is characterized by long face, upslanting palpebral fissures, epicanthal folds, large lax lips, high-arched palate, micrognathia, prominent nose, tall and thin body habitus, broad hands and feet, and varying degrees of mental retardation [White et al., 1995; Tonk et al., 2003]. At least 22 cases of 4p16.1–p12 deletion have been reported [reviewed by Tonk et al., 2003], 17 of whom had a 4p16.1–p14 deletion with a common clinical profile [Romain et al., 1985; Fryns et al., 1989; Davies et al., 1990; Ishikawa et al., 1990; Chitayat et al., 1995; White et al., 1995; Innes et al., 1999; Tonk et al., 2003] (Table I). Here we report a girl with mental retardation, overgrowth and mild facial anomalies, who has a de novo 46,XX,del(4)(p16.1p15.2). A well-proportioned overgrowth pattern in our patient seems distinctive in comparison to reported features of patients with the proximal 4p deletion syndrome.

The patient, a 24-year-old female Han Chinese, was born at full-term to a 26-year-old G1P1 mother who reported an unremarkable pregnancy and nondiabetic history. Consanguinity of the parents was denied. Family history was negative for tall habitus: the body weight/height of her father, mother and a sister was 67 kg/175 cm, 49 kg/165 cm, and 43 kg/160 cm, respectively. Birth weight of the patient was 3,550 g (75th centile), length 61 cm (>97th centile) and OFC 37 cm (>97th centile). She has always been taller than the Chinese age-cohorts since birth. She raised her head at age 6 months, spoke at 15 months and walked at 3 years. She was diagnosed in her early childhood to have mental retardation by a local pediatrician and never attended school except for kindergarten. She has been able to care for herself since she was a teenager. On physical examination at age 23 years, her height was 181 cm (>97th centile), weight 74 kg (>97th centile) and OFC 58 cm, facial length 19 cm, and she had the following facial abnormalities: square-jawed face, epicanthal folds, prominent nose with overhanging tip, short philtrum, high-arched palate and hypoplastic earlobes (Fig. 1a,b). She has a tall, thickset and proportionate habitus without broad hands and feet. Her carpal bone age was advanced during her childhood and adolescence, but the recent radiographic findings at age 24 years were normal. Her first menstruation appeared at age 12 years, then it came regularly, and her secondary sexual characteristics developed normally. Psychometric testing showed moderate mental retardation with estimated IQ of 50, with poorer performance in calculations. Clinical manifestations of the patient including her facial gestalt did not fit to those for any of generalized overgrowth syndromes, such

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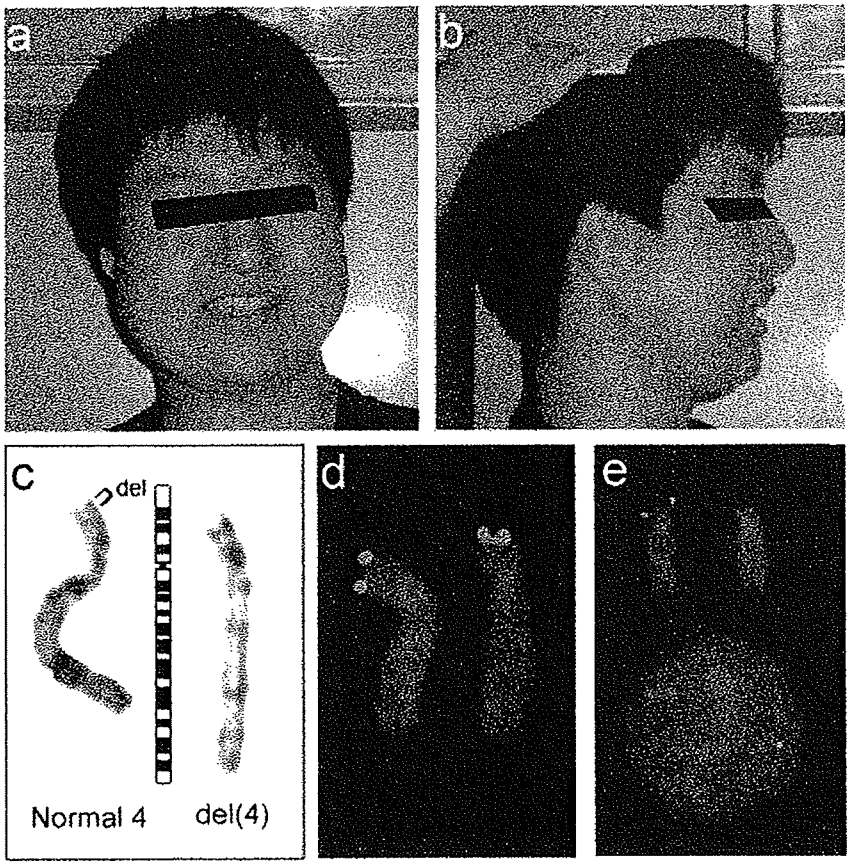

FIG. 1. A girl with a proximal 4p deletion. **a,b**: Facial appearance at age 23 years, showing well-developed, square-jawed face with hypoplastic earlobe. **c**: GTG-banded partial karyotype, showing del(4)(p16.1p15.2). **d,e**: FISH analysis using a BAC clone RP11-1150D2, showing signals in both normal and derivative chromosomes 4, and using a BAC clone RP11-29N16, showing a signal only in normal 4p and only one signal in an interphase cell.

as Weaver, Sotos, Simpson-Golabi-Behmel, Seip-Berardinelli, Perlman, Nevo, MOMO, Marshall-Smith, Beckwith-Wiedemann or Bannayan-Riley-Ruvalcaba syndromes [Douglas et al., 2003]. Thus, it is most likely that her overgrowth is constitutional and associated with a chromosomal deletion below.

High-resolution GTL-banding showed a 46,XX, del(4)(p16.1p15.2) karyotype (Fig. 1c). Fluorescence in situ hybridization (FISH) analysis with 11 BAC clones mapped to 4pter–4p14 [Kondoh et al., 2003] revealed that GS-36p21, RP11-1150D2 (Fig. 1d), 261G12 and 24K3 were retained, but RP11-29N16 (Fig. 1e), 77N9, 46O17, 79N22, 116N19, 192P23, and 106M4 were deleted. These results indicated that the WHS critical region is not deleted and that the proximal and distal deletion breakpoints are located in the regions between UCSC coordinate chromosome 4 nucleotide 24,549,727 and 24,551,523 and between nucleotide 6,504,169 and 6,504,249, respectively. Therefore, the deletion is assigned to an 18-Mb region (nt. 6,504,169–24,551,523) at 4p16.1–p15.2. The karyotypes of her father, mother and sister were normal.

Chitayat et al. [1995] reported three cases of the proximal 4p deletion syndrome and proposed 4p15.33–p15.2 as the minimal deleted segment for this syndrome, which was later supported by Innes et al. [1999]. However, our patient has a deletion encompassing the 4p16.1–p15.2 region, and there have been five reported cases of a deletion similar to our patients [Davies et al., 1990; White et al., 1995; Innes et al., 1999; Tonk et al., 2003]. All of these five cases shared several clinical features that include a long face, epicanthal folds, distinctive nose, thick lower lip, tall and thin habitus and moderate mental retardation. Thus, Tonk et al. [2003] suggested that the critical region for the proximal 4p deletion syndrome can be narrowed to a region from 4p16.1 to p15.2. As all reported cases that had a 4p16.1–p15.1 deletion manifested all typical features of this syndrome (Table I), the critical region should be confined to 4p16.1–p15.1. By a review of breakpoints of 4p16.1–p14 deletions in reported patients (Table I), we found that the tall habitus is most likely attributed to 4p16.1–p15.32 deletion, probably implying the presence of a negative control mechanism against tall status or overgrowth.

Overgrowth and other features in our patient merit comments. According to the information from Table II, 82% (14/17) of patients with a 4p16.1–p14