Phenotypic discordance is a common observation in complex genetic diseases, and a postzygotic somatic change is only one of several proposed mechanisms for discordance. Since CLP is a congenital defect, nongenetic intrauterine environmental factors such as unequal cell allocation at twinning and disproportionate placental blood supply may contribute to discordance (Gringras & Chen, 2001). If such developmental influences alone were responsible for MZ twin discordance, then one would expect an excess of CLP in MZ twins compared to singletons. This is not the case as studies in Denmark have demonstrated no significant difference in prevalence of CLP in twins versus singletons (Christensen & Fogh-Andersen, 1993a; 1993b). The absence of genomic differences between MZ twins underscores their use as matched case-controls particularly in studies exploring the environmental component in complex traits. With the availability of array-based techniques to simultaneously scan the whole genome, it is now possible to map genomic alterations at a high resolution and additional experimental tools such as quantitative PCR and multiplex ligation-dependent probe amplification (MLPA) can be applied to independently confirm any observed genomic alterations. This straightforward approach can be applied to other cases of discordant MZ twins where identification of genomic alterations can reveal potential candidate genes, or provide evidence of a gene's involvement in disease etiology.

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Original Article

Human Papillomavirus DNA in Plasma of Patients with HPV16 DNA-positive Uterine Cervical Cancer

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Objectives: The squamous cell carcinoma antigen is considered the most accurate serologic tumor marker for uterine cervical carcinoma. However, serum squamous cell carcinoma antigen levels were found to correlate significantly with clinical severity of atopic dermatitis and chronic renal failure. The present study was conducted in patients with human papillomavirus 16 DNA-positive uterine cervical cancer to determine the plasma level of human papillomavirus 16 DNA and the diagnostic values of plasma human papillomavirus DNA in these patients.

Methods: Forty-three human papillomavirus 16-positive patients with cervical intraepithelial neoplasia or uterine cervical squamous cell carcinoma were recruited in this study. The diagnosis was cervical cancer in 20 patients, high-grade squamous intraepithelial lesions in 21, low-grade squamous intraepithelial lesions in 1 and negative for intraepithelial lesion or malignancy in 3 patients. Before any treatment, blood samples were collected from all patients. For analysis of human papillomavirus DNA in plasma of patients with cervical cancer, quantitative polymerase chain reaction fluorescent assay for human papillomavirus 16 was performed using human papillomavirus 16 primers and SYBR Green dye using the LightCycler 480 SW1.5 apparatus.

Results: Plasma human papillomavirus 16 DNA was detected in only 30.0% of the patients with human papillomavirus 16-positive cervical cancer and in none of normal controls. The copy number of plasma human papillomavirus 16 DNA was higher in patients with invasive cancer than in those with cervical intraepithelial neoplasia (CIN3), micro-invasive cancer and in normal individuals.

Conclusions: These results indicated that the plasma human papillomavirus DNA level could be potentially used as a marker of low-invasive cervical cancer tumors in patients with normal squamous cell carcinoma antigen levels before treatment.

Key words: gynecol-basic — genetics-cancer genetics — diagnosis

INTRODUCTION

The squamous cell carcinoma antigen (SCCA), an SCC tumor-associated protein, was first discovered in uterine cervical SCC by Kato and Torigoe (1) in 1977. SCCA is expressed not only in SCC but also in normal squamous epithelium (2) and considered the most accurate serological marker of uterine cervical carcinoma. However, serum levels of SCCA have been reported also to correlate with the

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clinical severity of atopic dermatitis, chronic hypertension and chronic renal failure (3).

Epidemiological studies have provided data on the incidence of human papillomavirus (HPV) infection and the risk factors for HPV infection and genital precancerous lesions (4–6). The long duration of HPV infection is attributed to the ability of the virus to subvert innate immune responses (7,8). Persistent infection with HPV confers a strong risk for the development of subsequent neoplasia (9,10). Several groups have examined the prevalence of HPV DNA in plasma of cervical cancer patients, although there is discrepancy in the results of these studies (11–14). The different prevalence rates could be due to the different populations examined, different HPV types and different methods used for the detection.

The present study was designed to determine the prevalence of HPV16 DNA in plasma of patients with HPV16 DNA-positive uterine cervical cancer and its diagnostic and prognostic value in these patients.

PATIENTS AND METHODS

Patients and Sample Collection

This study was approved by the Human Ethics Committee of Nagasaki University Hospital. Forty-three HPV16-positive patients with cervical intraepithelial neoplasia (CIN) or uterine cervical SCC were recruited in this study. All patients were examined and/or treated at Nagasaki University Hospital between December 2007 and June 2008. Each patient underwent pelvic examination followed by conventional cervical cytology and determination of the serum SCCA level (see below). After obtaining informed consent, specimens for HPV typing were harvested (SurePath and CytoRoch, MBL) and evaluated for HPV DNA by polymerase chain reaction (PCR).

Table 1 shows the stage of cervical cancer and treatment modalities. The diagnosis was cervical cancer in 20 patients, high-grade squamous intraepithelial lesions in 21, low-grade squamous intraepithelial lesions in 1 and negative for intraepithelial lesion or malignancy (NILM) in 3 patients. The stage of cervical cancer was CC Ia1 in 4 patients, CC Ia2 in 1, CC Ib1 in 2, CC Ib2 in 2, CC IIa in 4, CC IIb in 4, CC IIIb in 1, CC IVa in 1 and CC IVb in 1, according to the International Federation of Gynecology and Obstetrics (FIGO) staging system. We also recruited 20 normal individuals who had no history of cervical dysplasia or neoplasia, as negative controls. Subjects of the control group were confirmed by cervical cytology to be free of cancer or intraepithelial lesions and by hybrid Capture II test to be HPV DNA-negative. Before any treatment, 7 ml blood samples were collected from all patients and placed immediately into tubes containing ethylenediaminetetraacetic acid.

SCCA Assay

Serum SCCA levels were assayed by radioimmunoassay (RIA, SRL, Tokyo). The normal value of SCCA in our hospital is ≤ 1.5 ng/ml.

Table 1. Background, treatment and prognosis of patients with cervical cancer

Patient	Age	FIGO staging	Treatment	Pathology	Prognosis
CC1	20	Ia1	Conization	SCC	CR
CC2	42	Ia1	Modified radical hysterectomy	SCC	CR
CC3	64	Ia1	Modified radical hysterectomy	SCC	CR
CC4	41	Ia1	Modified radical hysterectomy	SCC	CR
CC5	43	Ia2	Modified radical hysterectomy	SCC	CR
CC6	49	Ib1	Radical hysterectomy	SCC	CR
CC7	83	Ib1	Radiotherapy	SCC	CR
CC8	53	Ib2	Chemoradiotherapy	SCC	CR
CC9	42	Ib2	Radical hysterectomy[a]	SCC	CR
CC10	61	IIa	Chemoradiotherapy	SCC	CR
CC11	65	IIa	Radical hysterectomy[b]	SCC	CR
CC12	54	IIa	Radical hysterectomy[c]	SCC	CR
CC13	62	IIa	Chemoradiotherapy	SCC	CR
CC14	45	IIb	Chemoradiotherapy	SCC	CR
CC15	61	IIb	Not clear[d]	SCC	Not clear
CC16	60	IIb	Chemoradiotherapy	SCC	CR
CC17	56	IIb	Chemoradiotherapy	SCC	CR
CC18	80	IIIb	Radiotherapy	SCC	CR
CC19	65	Iva	Radiotherapy	SCC	PR
CC20	43	IVb	Chemoradiotherapy	SCC	PR

FIGO, The International Federation of Gynecology and Obstetrics; SCC, squamous cell carcinoma; CR, complete response to therapy and no recurrence or metastasis; PR, partial response.
[a]Patient received chemoradiotherapy due to invasion of blood vessels after surgery.
[b]Patient received chemoradiotherapy after operation.
[c]Patient received Neo-adjuvant chemotherapy before operation.
[d]Treatment and prognosis are not clear due to changes in hospitals.

Isolation of DNA

Blood samples were centrifuged at 3000 rpm for 5 min at room temperature. The plasma was saved and stored at $-20°C$ until analysis. The DNA was extracted from 200 μl of plasma using a commercial kit (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany) and the DNA was eluted with 50 μl of MilliQ.

Quantitative Real-time PCR Analysis of HPV DNA

For analysis of HPV DNA in plasma of patients with cervical cancer, quantitative real-time PCR (qPCR) fluorescent assay for HPV16 was performed using HPV16 primers and

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SYBR Green dye using the LightCycler 480 SW1.5 apparatus (Roche Molecular Biochemicals). HPV16 primers were as follows. (i) HPV16E6E7 forward primer: 5′-ATC ATC AAG AAC ACG TAG AG-3′; reverse primer: 5′-GAT CAG TTG TCT CTG GTT GCA AAT-3′. (ii) HPV16E7 forward primer: 5′-CAGCTCAGAGGAGGAGGATG-3′; reverse primer: 5′-ATTGTAATGGGCTCTGTCCG-3′. (iii) HPV16E6 forward primer: 5′-ACTGCAATGTTTCAGGACCC-3′; reverse primer: 5′-GCATAAATCCCGAAAAGCAA-3′. The qPCR was set up in a reaction volume of 20 μl. Each qPCR contained, in 1 μl of total plasma DNA, 2 μl of 5 μM each forward and reverse primers, 10 μl of 2× Quantitect SYBR Green PCR Master Mix (Qiagen). For amplification of HPV16E6E7, the qPCR setting was initial denaturation at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 10 s, annealing and extension at 60°C for 1 min. For amplification of HPV16E6 and HPV16E7, the qPCR setting was initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 10 s, annealing at 58°C for 1 min and extension at 72°C for 1 min.

Determination of Viral Load

To prepare plasmids containing HPV16E6 and/or HPV16E7 sequences for standard curves, we used the HPV16-positive cervical cancer cell line (Caski cell). Total cellular DNA was isolated by using Genomic DNA purification kit (Promega, Madison, WI, USA) according to the instructions provided by the manufacturer. HPV16E6 and/or HPV16E7 were re-cloned in PCR2.1-TOPO for standard curves. From the known molecular weights of the recombinant plasmids, the amount of plasmid DNA equivalent to 5×10^{10} copies of HPV16E6 and/or HPV16E7 was first determined. Standard curves were generated automatically by plotting the threshold cycle values against the logarithm of the copy numbers of plasmid DNA standards (serial 10-fold dilution from 5×10^{10} to 0.5 copies of HPV16E6 and/or HPV16E7). The copy numbers of each sample were calculated from the standard curve. The concentration of plasma HPV DNA was expressed as copies of HPV genome per milliliter of plasma. The results are expressed as mean ± SD ($n = 3$).

Statistical Analysis

The Mann–Whitney test was used to compare the HPV DNA copy numbers among cancers of different stages. Probability values <0.05 were regarded as statistically significant.

RESULTS

Quantitative PCR was highly sensitive in detection of HPV16 DNA with as little as 50 copies/μl. Plasma HPV16E6E7 DNA was detected in 6 of 20 (30.0%) patients with HPV16-positive invasive cervical cancer, but in none of those with CIN3 and normal controls (Table 2). The copy numbers of plasma HPV16E6E7 DNA in patients with invasive cancer were higher than in micro-invasive cancer, CIN3 and normal individuals (Table 2).

Table 2. Incidence of plasma HPV16E6E7 DNA according to the stage of cervical cancers and detection levels of plasma HPV16E6E7 DNA in cervical cancer patients and precancerous disease

	Cases	Positive	Plasma HPV16E6E7 DNA (copies/ml of plasma)		
			Frequency (%)	Maximum	Median
Normal	20	0	0	0	0
NILM	3	0	0	0	0
L-SIL	1	0	0	0	0
H-SIL	20	0	0	0	0
Cancer (FIGO stage)					
Ia1	4	1	25	507	300
Ia2	1	0	0	0	0
Ib1	2	0	0	0	0
Ib2	2	0	0	0	0
IIa	4	1	25	96 583	96 583
IIb	4	2	50	342 833	186 624
IIIb	1	0	0	0	0
IVa	1	1	100	229 792	135 381
IVb	1	1	100	17 768	11 935

HPV, human papillomavirus; normal, negative control; NILM, negative for intraepithelial lesion or malignancy; L-SIL, low-grade squamous intraepithelial lesion; H-SIL, high-grade squamous intraepithelial lesion.

Table 3 shows the plasma HPV16E6E7 DNA viral loads, disease stage and serum levels of SCCA. Only 4 out of 13 (30.8%) patients with high serum SCCA levels had detectable levels of plasma HPV16E6E7 DNA before operation. On the other hand, 2 out of 7 (28.6%) patients with normal serum SCCA levels had detectable levels of plasma HPV16E6E7 DNA before operation.

We also checked plasma HPV16 DNA using other primers, including HPV16E7 forward primer and HPV16E7 reverse primer to detect part of E7 (70 bp) as well HPV16E6 forward primer and HPV16E7 reverse primer to detect part of E6 (159 bp), to rule out differences in HPV DNA detection rate in the same patient based on differences in primers' position (Table 3). HPV16E6 and/or HPV16E7 DNA were detected in the plasma of two to three out of six patients with cervical cancer who were positive for HPV16 DNA by the HPV16E6E7 primer.

DISCUSSION

The major finding of the present study was the detection of HPV16 DNA in 60% of primary cervical cancers and in plasma samples of 30.0% of the patients with HPV16 DNA-positive primary tumors.

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Table 3. Pre-treatment plasma HPV16 DNA levels and SCCA levels in HPV16-positive patients with cervical cancer

Patient	Diagnosis	SCCA	Copies/ml of plasma		
			HPV16E6E7	HPV16E7	HPV16E6
CC1	Ia1	2.1	0	0	0
CC2	Ia1	0.6	0	0	0
CC3	Ia1	0.9	0	0	0
CC4	Ia1	1.3	300	0	0
CC5	Ia2	1.7	0	0	0
CC6	Ib1	1.1	0	0	0
CC7	Ib1	1.3	0	0	0
CC8	Ib2	14.3	0	0	0
CC9	Ib2	9.9	0	0	0
CC10	IIa	3.0	96 583	334 000	0
CC11	IIa	2.6	0	0	0
CC12	IIa	2.7	0	0	0
CC13	IIa	1.7	0	0	0
CC14	IIb	12.9	0	0	0
CC15	IIb	9.3	342 833	129 000	1 190 000
CC16	IIb	18.2	0	0	0
CC17	IIb	1.5	30 417	406 000	556 667
CC18	IIIb	1.4	0	0	0
CC19	Iva	35.9	135 381	0	0
CC20	IVb	12.2	11 935	0	0

SCCA, squamous cell carcinoma antigen.

The detection rate of HPV DNA in plasma of patients with uterine cervical cancer remains controversial. Pornthanakasem et al. (11) detected HPV DNA in the plasma of 12% of their HPV-positive patients with cervical cancer, whereas the same rate was 70% in another study (12). Furthermore, Yang et al. (13) reported detection of HPV16 DNA in plasma samples of 50% of their patients with cervical cancer. In this context, Capone et al. (15) reported that they could not detect HPV16 DNA in the plasma of 65 patients with nasopharyngeal SCC using L1 primer but detected the same DNA in the plasma of 2 of their 65 patients by real-time PCR using E7 primer. These differences in the detection rate may be due to differences in sample numbers and method of analysis.

In the present study, plasma HPV16E6E7 DNA was detected in 6 of 20 (30.0%) HPV16-positive patients with invasive cervical cancer but in none of the normal controls. The copy number of plasma HPV16 DNA in patients with invasive cancer was higher than in CIN3 and micro-invasive cancer and in normal individuals. On the other hand, the copy number of plasma HPV16 E6E7 DNA in cervical cancer stage IVa was higher than that of cervical cancer stage IVb. More sample collection and further studies are required to determine the relationship between the detection level of HPV DNA in plasma and clinical stage of uterine cervical cancer.

The source of plasma HPV DNA remains unclear. However, the DNA level is probably related to tumor size, stage and presence or absence of metastasis. In this regard, Pornthanakasem et al. (11) reported that the plasma level of HPV DNA in metastatic patients was three times higher than that of patients without metastasis. Their results suggested that the amount of plasma HPV DNA could be a useful tumor marker for prediction of disease progression and clinical outcome after treatment of patients with cervical cancer.

In our study, only 30.8% of the patients with high levels of serum SCCA were plasma HPV16 DNA-positive. And detection rate of plasma HPV16 DNA was different based on differences in primers' position (Table 3). In this context, the form of HPV DNA in the plasma of cervical cancer patients is not clear. It is possible that various HPV DNA fragments are present in the peripheral circulation since the HPV DNA detection rates in the plasma varied with the use of different primers in the same patients with cervical cancer.

What is the source of HPV DNA in the peripheral circulation? While the exact source is not clear at this stage, circulating HPV DNA levels could represent lysis of circulating cancer cells or micrometastasis shed from the tumor (16,17). Others have suggested that circulating tumor DNA in plasma might reflect tumor cell metastasis because of the high *in vitro* transforming activity (18,19).

The results of the present study indicated that HPV16E6E7 DNA in plasma might not be a sensitive marker of cervical cancer recurrence because plasma HPV16E6E7 DNA before operation was detected in only 4 out of 13 (30.8%) patients with high serum SCCA levels. However, HPV DNA could be potentially used as a marker of low-invasive cervical cancer tumors in patients with normal SCCA levels before treatment because two out of seven (28.6%) patients with normal serum SCCA levels had detectable levels of plasma HPV16E6E7 DNA before operation. We recommend the use of several HPV DNA primers to detect various HPV DNA fragments in the peripheral circulation in patients with cervical cancer.

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Conflict of interest statement

None declared.

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human reproduction

CASE REPORT *Reproductive genetics*

A case of Kallmann syndrome carrying a missense mutation in alternatively spliced exon 8A encoding the immunoglobulin-like domain IIIb of fibroblast growth factor receptor 1

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ABSTRACT: Fibroblast growth factor receptor 1 (*FGFR1*) is one of the causative genes for Kallmann syndrome (KS), which is characterized by isolated hypogonadotropic hypogonadism with anosmia/hyposmia. The third immunoglobulin-like domain (D3) of *FGFR1* has the isoforms *FGFR1-IIIb* and *FGFR1-IIIc*, which are generated by alternative splicing of exons 8A and 8B, respectively. To date, the only mutations to have been identified in D3 of *FGFR1* are in exon 8B. We performed mutation analysis of *FGFR1* in a 23-year-old female patient with KS and found a missense mutation (c.1072C>T) in exon 8A of *FGFR1*. The c.1072C>T mutation was not detected in her family members or in 220 normal Japanese and 100 Caucasian female controls. No mutation in other KS genes, KS 1, prokineticin-2, prokineticin receptor-2 and *FGF-8* was detected in the affected patient or in her family members. Therefore, this is the first case of KS carrying a *de novo* missense mutation in *FGFR1* exon 8A, suggesting that isoform *FGFR1-IIIb*, as well as isoform *FGFR1-IIIc*, plays a crucial role in the pathogenesis of KS.

Key words: Kallmann syndrome / *FGFR1b mutation* / fibroblast growth factor receptor 1 isoform expression

Introduction

Kallmann syndrome (KS), which is characterized by isolated hypogonadotropic hypogonadism (IHH) and anosmia/hyposmia, is a clinically and genetically heterogeneous disorder. To date, five causative genes for KS have been reported: KS 1 (*KAL1*, GenBank accession M97 252), prokineticin-2 (*PROK2*, GenBank accession NM 021935), prokineticin receptor-2 (*PROKR2*, GenBank accession NM 144773), fibroblast growth factor-8 (*FGF-8*, GenBank accession NM 033163) and fibroblast growth factor receptor 1 (*FGFR1*, GenBank accession NM 023110.2).

Although sporadic cases of KS are more frequent, families with KS have been reported with X-linked recessive or autosomal dominant or recessive modes of inheritance. Mutations in *KAL1* have been found in familial cases with X-linked recessive inheritance (Franco *et al.*, 1991; Legouis *et al.*, 1991). Mutations in *PROK2* were detected in the heterozygous state, whereas *PROKR2* mutations were found in the heterozygous, homozygous or compound heterozygous state (Dodé *et al.*, 2006). *PROKR2/PROK2* mutations with true pathogenic potential were found only in the homozygous state (Abreu *et al.*, 2008), and any dominant-negative effect of *PROKR2* mutations was ruled out (Monnier *et al.*, 2009). Mutations in *FGFR1* or *FGF8* underlie an autosomal dominant form with incomplete penetrance. Therefore, KS families harbouring heterozygous *FGFR1* or *FGF8* mutations display variable olfactory phenotypes (Dodé *et al.*, 2003; Falardeau *et al.*, 2008), and a few cases with heterozygous *FGFR1* mutations show a normosmic IHH (Pitteloud *et al.*, 2006a). The *FGFR1* gene, which is located on chromosome 8p12, comprises 18 exons (Ruta *et al.*, 1989), and various mutations, including missense and protein truncation mutations, have been reported (Trarbach *et al.*, 2007). The third immunoglobulin-like domain (D3) of *FGFR1* has the isoforms *FGFR1-IIIb* and *FGFR1-IIIc*, which are generated by alternative splicing

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of exons 8A and 8B, respectively (Johnson *et al.*, 1991). To date, mutations in D3 of *FGFR1* have only been identified in exon 8B, which encodes immunoglobulin domain IIIc, suggesting that isoform FGFR1-IIIc plays a crucial role in the pathogenesis of KS (Pitteloud *et al.*, 2006b; Trarbach *et al.*, 2006; Dodé *et al.*, 2007).

Here, we report for the first time a KS case carrying a *de novo* missense mutation in the alternatively spliced exon 8A of *FGFR1-IIIb*.

Materials and Methods

Patient and family

Patient (Subject II-2) was a 23-year-old Japanese woman. When she was 18 years old, she was treated at Nagasaki University Hospital because of primary amenorrhea with anosmia. Her height was 159.2 cm and her weight was 72.0 kg. Her serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estradiol (E2) were less than 0.5 and 1.5m IU/ml and 10 pg/ml, respectively. Her LH frequent sampling study (sampling performed every 15 min) showed a low-amplitude pattern of LH pulsation (Fig. 1). Her brain magnetic resonance imaging (MRI) examination was negative for tumors and showed no anatomical abnormalities of the hypothalamic–pituitary region and olfactory bulbs. A scratch-and-sniff test (UPSIT, Sensonics, Haddon Hts, NJ, USA) (Doty *et al.*, 1985), which determines ability to smell, indicated anosmia. She was diagnosed as having KS and received hormone replacement therapy for 5 years. Her mother (Subject I-1) was normosmic and had normal puberty and regular menstrual cycles. Her father (Subject I-2), elder brother (Subject II-1) and younger brother (Subject II-3) were also normosmic and had normal puberty (Fig. 2).

Molecular analysis

DNA extraction

Whole blood samples were obtained from the KS patient and from her mother, father, elder and younger brothers. All samples were collected after obtaining written informed consent and the study protocol was approved by the Institutional Review Board of Nagasaki University. Genomic DNA from lymphocytes was extracted using a QIAamp DNA blood mini kit (Qiagen, Düsseldorf, Germany), according to the manufacturer's instructions.

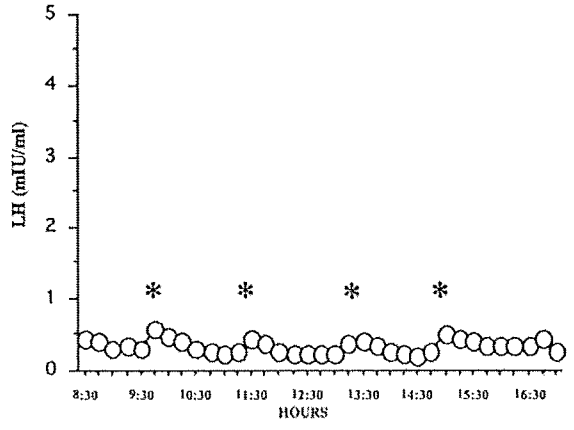

Figure 1 LH pulsation pattern in a case of KS, assayed using an LH frequent sampling study. LH frequent sampling was performed every 15 min. *Low-amplitude pattern of LH pulse.

Sequence analysis

FGFR1 consists of 18 coding exons. Intragenic mutations were investigated by PCR amplification and sequence analysis using 14 pairs of primers, as previously described (Dodé *et al.*, 2003; Sato *et al.*, 2004). Genomic DNA was PCR amplified using conditions of 95°C for 12 min followed by 95°C for 30 s, 59°C for 30 s and 72°C for 60 s for 35 cycles and a final cycle of 72°C for 10 min. PCR products were analyzed by agarose gel electrophoresis, purified with ExoSAP-IT and subjected to sequencing reactions. Sequencing reactions were performed using the BigDye terminator v.3.1 kit and analyzed with an ABI PRISM 3100 Genetic Analyzer™ (Applied Biosystems). The KS patient carrying a mutation in *FGFR1* and her family members were also screened for mutations in the other genes known to be involved in KS [*KAL1*, *PROK2*, *PROKR2* and *FGF8*]. Whether the mutation leads to a change in the protein structure and function was predicted bioinformatically using the ExPASy proteomics server (http://au.expasy.org/) and PolyPhen (http://genetics.bwh.harvard.edu/pph/).

Confirmation of the alternatively spliced exon

Isolation of a full-length murine *Fgfr1-IIIb* showed that Fgfr1-IIIb was a transmembrane receptor (Beer *et al.*, 2000). Although the mRNA encoding exon IIIb has been found in human (Johnson *et al.*, 1991), the presence of sequences encoding the intracellular domain has not yet been demonstrated. Therefore, to determine the splice site of exon 8A and to detect *FGFR1-IIIb* mRNA encoding the intracellular domain, we performed RT–PCR using specific primers to amplify the splice isoform containing exon 8A. Kal23 is designed to span exons 7 and 8A for specific annealing to the *FGFR1-IIIb* isoform, which is spliced from exon 7 to exon 8A (Fig. 3A). Kal5 is designed within exon 8B for specific annealing to the *FGFR1-IIIc* isoform, which is spliced from exon 7 to exon 8B. Kal2 and Kal6 are designed within exons 7 and 9, respectively, for annealing to the D3 isoforms of *FGFR1*. Primer sequences were as follows: kal2: 5′-GACAGAAGGTCGGTTATGTC-3′, Kal23: 5′-CAGATCTTGAAGC ATTCGGG-3′, Kal5: 5′-GGTGGTATTAACTCCAGCAG-3′ and Kal6: 5′-GTACAGGGGCGAGGTCATCA-3′. The BD multiple tissue complementary DNA (cDNA, MTC) panels Human I and Human II (BD Biosciences Clontech, Mountain View, CA, USA) were used to detect the expression of each isoform of *FGFR1*. PCR amplification was performed on cDNAs as follows: 94°C, 30 s; 62°C, 30 s; 72°C, 1 min; 40 cycles. PCR products were analyzed by agarose gel electrophoresis and sequenced using then ABI PRISM 3100 Genetic Analyzer™.

Results

Sequence analysis of the entire coding region of *FGFR1*, including exon–intron boundary regions, showed that the KS patient had a mutation (c.1072C>T) in exon 8A of *FGFR1-IIIb*, while the other family members did not (Fig. 2). However, the full-length *FGFR1* mRNA that includes exon 8A is not deposited in the full-length cDNA database (GenBank accession no. NM 023110.2). RT–PCR analysis indicated that most transcripts containing exon 8A were spliced to exon 8B in all adult tissues except bone marrow (data not shown). We wished to demonstrate the existence of an alternative transcript, exon 8A which was spliced to exon 9 encoding the transmembrane helix; therefore, RT–PCR products from human fetal brain were cloned and sequenced. In 1 of 27 clones exon 8A was spliced to exon 9 (designated here '*FGFR1-IIIb*', GenBank accession FJ809917, see Fig. 3B), while in the other clones exon 8A was spliced to exon 8B (designated here

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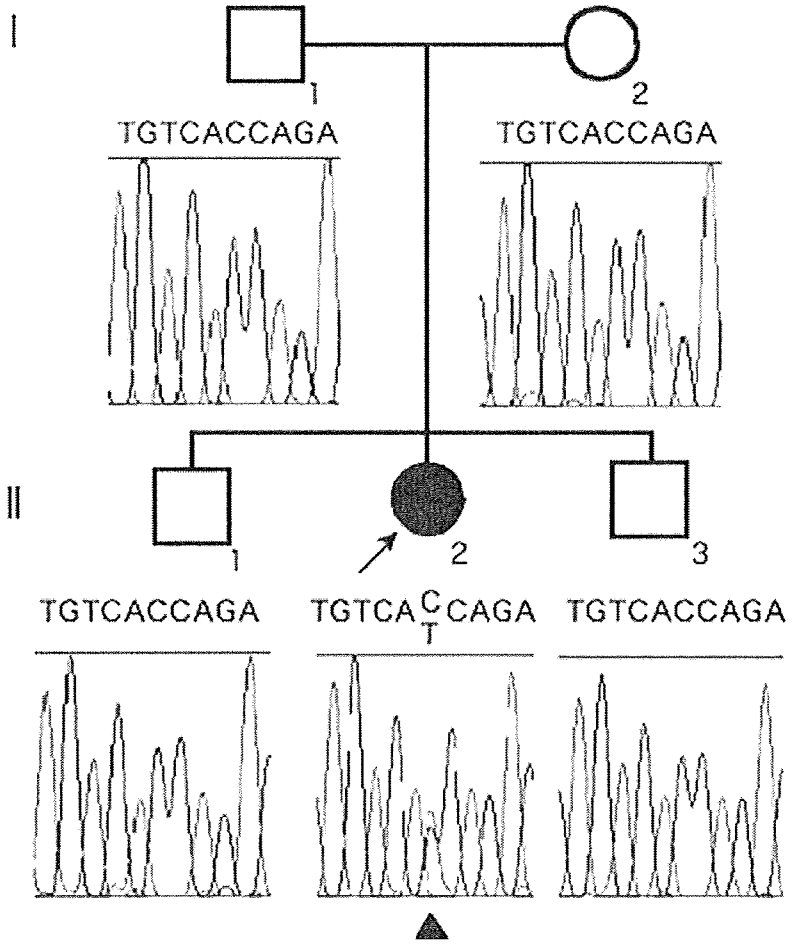

Figure 2 Pedigree of patient's family and the results of fibroblast growth factor receptor 1 (*FGFR1*) sequence analysis. II-2 is a 23-year-old woman with KS. The patient had a mutation in *FGFR1* (C>T) but the other family members did not. The mutation was *de novo* because parentage was assured. Arrowhead under the electropherogram indicates the mutation site.

'*FGFR1-secr*', GenBank accession FJ809916, see Fig. 3B). The exact acceptor and donor sites of exon 8A in '*FGFR1-IIIb*' mRNA, which produces a membrane-bound FGFR1-containing D3, were determined by sequence analysis of splice isoforms, '*FGFR1-IIIb*' and '*FGFR1-secr*', (Fig. 3). As most full-length *FGFR1* cDNAs in the database were transcripts containing exon 7–exon 8B–exon 9 (designated here '*FGFR1-IIIc*', GenBank accession NM 023110.2, see Fig. 2B) without exon 8A, '*FGFR1-IIIc*' is likely to be the most abundantly expressed human isoform. The EST, CA488712.1, was the only isoform in the EST database corresponding to '*FGFR1-IIIb*'. Although both '*FGFR1-IIIb*' and '*FGFR1-IIIc*' encode membrane-bound *FGFR1*, '*FGFR1-secr*' encodes a secreted form of *FGFR1* because of a sequence frameshift and a termination codon in exon 9.

The exons 8A and 8B of the human FGFR1 isoforms shared the amino acid sequence at 354–357; WLTV. However, exon 8A ends with six extra amino acids at 358–363, TRPVAK, whereas exon 8B ends with only two, LE. These sequences are identical in the mouse Fgfr1 isoforms (Beer *et al.*, 2000). The mutation in exon 8A of *FGFR1-IIIb* (GenBank accession no. FJ809917 bankit1193625) is c.1072C>T at the cDNA level and p.T358I at the amino acid level. Bioinformatic analysis shows the mutated amino acid residue to be conserved between human and mouse and to be located in D3 of *FGFR1-IIIb*, which is a critical region for FGF ligand binding. However, the mutation was not predicted to produce a change in the human protein structure. The c.1072C>T mutation was not detected in 220 normal Japanese women or in 100 normal Caucasian women. The patient had no mutation in any of the other four KS genes.

Discussion

Mouse *Fgfr1-IIIb* has a low level of expression in a wide variety of adult tissues, but a high level of expression in skin and brain, indicating the existence of specific splicing factors in skin and brain that recognize the relatively weak *Fgfr1-IIIb* splice site (Beer *et al.*, 2000). Consistent with the expression pattern of mouse *Fgfr1-IIIb*, we could isolate human *FGFR1-IIIb* from a fetal brain cDNA library but not from adult

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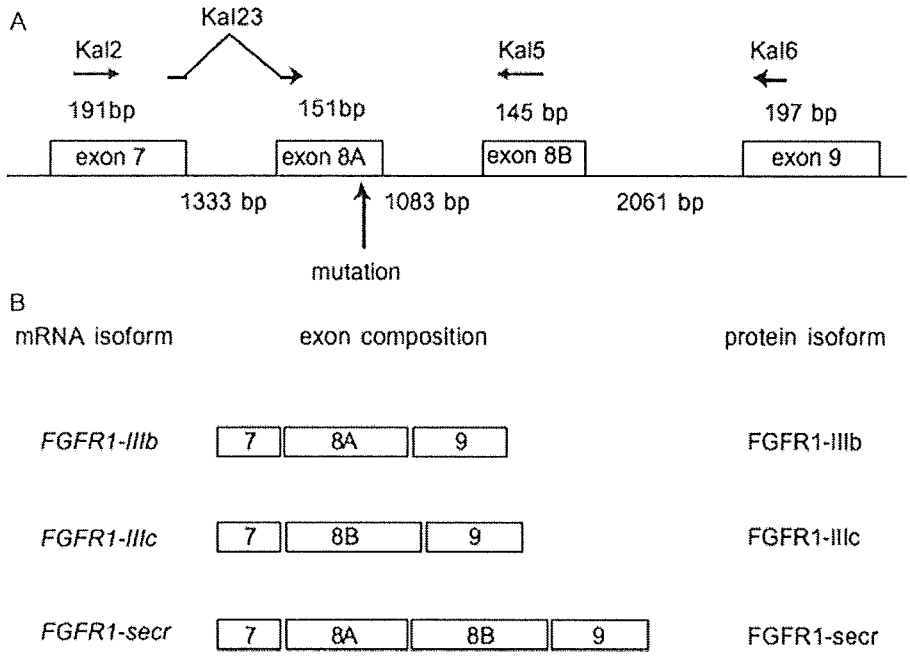

Figure 3 Genomic organization of *FGFR1* around exon 8A. (A) The numbers with bp indicate exon length (over the line) and intron length (under the line). The mutation is located near the end of exon 8A. Horizontal arrows indicate the locations of primers used to perform RT–PCR amplification of the isoform containing exons 7 and 8A. Primer Kal23 is designed to span exons 7 and 8A for specific annealing to the *FGFR1-IIIb* isoform, which is spliced from exon 7 to exon 8A (Fig. 3A). Kal5 is designed within exon 8B for specific annealing to the *FGFR1-IIIc* isoform, which is spliced from exon 7 to exon 8B. Kal2 and Kal6 are designed within exons 7 and 9, respectively, for annealing to the D3 isoforms of *FGFR1*. Vertical arrow indicates mutation site. (B) Composition of mRNA isoforms and of putative protein structures. FGFR1-IIIb: membrane-bound form of FGFR1 with immunoglobulin-like domain IIIb encoded by exon 8A, FGFR1-IIIc: membrane-bound form of FGFR1 with immunoglobulin-like domain IIIc encoded by exon 8B, FGFR1-secr: a secreted form of FGFR1.

tissues. Most full-length *FGFR1* cDNAs in the database represent *FGFR1-IIIc*. *FGFR1-IIIc* is expressed at high levels, but *FGFR1-IIIb* is expressed at very low levels (Johnson *et al.*, 1991). We can amplify only a tiny amount of *FGFR1-IIIb* that has exon 8A spliced to exon 9 by RT–PCR using the primers Kal23 and Kal6. Most of the sequenced RT–PCR products corresponded to *FGFR1-secr*, suggesting that the expression level of the three isoforms is '*FGFR1-IIIc*' ≫ '*FGFR1-IIIsecr*' ≫ '*FGFR1-IIIb*' (Fig. 3B).

Several studies suggested that mutations in exon 8B of isoform *FGFR1-IIIc* are implicated in the pathogenesis of KS (Pitteloud *et al.*, 2006b; Trarbach *et al.*, 2006; Dodé *et al.*, 2007). Mice homozygous for alleles with a stop codon in exon IIIc displayed phenotypes resembling those of embryos homozygous for null alleles, while mice carrying an in-frame stop codon in exon IIIb were viable and fertile (Partanen *et al.*, 1998). Therefore, *Fgfr1-IIIc* is the dominant isoform that carries out the majority of the biological functions of the *Fgfr1* gene, whereas *Fgfr1-IIIb* plays a minor and to some extent redundant role (Partanen *et al.*, 1998). A receptor-binding analysis revealed no difference in the binding specificity between the endogenous *Fgfr1-IIIb* and an artificially created *Fgfr1-IIIb*, which had two different amino acids in the 3′-end of the unique IIIb exon (Beer *et al.*, 2000), suggesting that the carboxyl terminus of D3 may not overtly influence binding specificity. However, being expressed at low levels does not imply that '*FGFR1-IIIb*' has an unimportant role. The mutation we found, p.T358I, was located in exon 8A of *FGFR1-IIIb*. Therefore, p.T358I may affect ligand binding and cause the KS phenotype, although how this mutation affects the loss of function of *FGFR1-IIIb* is unknown. The spatio-temporal expression of any gene involved in development is key; therefore, the expression and functional involvement of *FGFR1-IIIb* may be important in the early embryonic brain, in particular during GnRH neuronal development. KS missense mutations in *FGFR1* are distributed in the first, second and third immunoglobulin-like domains (D1–D3), in the tyrosine kinase domain and also in the intracellular domain (Dodé *et al.*, 2003; Sato *et al.*, 2004; Albuisson *et al.*, 2005; Pitteloud *et al.*, 2006a; Trarbach *et al.*, 2006; Dodé *et al.*, 2007); therefore, the membrane-bound form of *FGFR1* is probably important for the KS phenotype. The membrane-bound form of *FGFR1-IIIb* could, therefore, be the critical isoform for the KS phenotype.

We present here, for the first time, a case of KS carrying a missense mutation in exon 8A of *FGFR1*, suggesting that the minor isoform '*FGFR1-IIIb*' as well as the major isoform '*FGFR1-IIIc*' has a crucial role in the pathogenesis of KS. Therefore, immunoglobulin-like domain IIIb may have an essential role in GnRH neuronal migration, which is initiated from the nasal placode and runs towards the forebrain following the olfactory sensory neuron axonal connection with the developing olfactory bulb. Further experiments are needed to show that the mutation in

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exon 8A causes KS, such as expression of the mutated isoform in transfected cells to analyze receptor stability and signaling efficiency. Although *FGFR1* containing immunoglobulin-like domain IIIb has not been analyzed intensively, our mutation report should encourage researchers to analyze immunoglobulin-like domain IIIb function and the spatio-temporal expression of exon 8A in fetal brain development.

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Vaginal delivery and cesarean section: comparison of maternal outcome in placental abruption with fetal death.

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要　旨

目的：胎児死亡を伴う常位胎盤早期剥離（早剥）において、分娩様式の違いによって母体予後に差があるか否か検討した。

方法：当院で胎児死亡を伴う早剥と診断された19例を対象とした。ただちに帝王切開術を行った5例（帝切群）、最初に経腟分娩を試みたが後に帝王切開術に変更した4例（変更群）、および経腟分娩を完遂した10例（経腟群）、以上3群について母体予後を検討した。

結果：母体年齢および分娩週数、妊娠合併症、入院時の頸管開大度、血液検査所見、分娩時出血量は3群間に差がなかった。診断から分娩までの時間は経腟群で帝切群と比較して分娩までの時間が長かった。輸血を要した例は、帝切群＋変更群で全例、経腟群で5/10例であった。

考察：今回の検討から、胎児死亡を伴う早剥例に対し、帝王切開術と経腟分娩いずれがよいかを判断することはできなかった。しかし、多くの例で安全に経腟分娩を完遂することが可能であった。

ABSTRACT

Objective: This study investigated whether the mode of delivery was associated with maternal prognosis in placental abruption with fetal death.

Method: Between January 1994 and March 2008, there were 19 cases of placental abruption with fetal death. We investigated the relationship between the mode of delivery and maternal prognosis. Five of 19 cases were delivered by cesarean section (group 1), 4 were delivered by cesarean section after attempting vaginal delivery (group 2), 10 had a vaginal delivery (group 3).

Result: There was no significant difference between the 3 groups for maternal age, gestational age at delivery, complications during pregnancy, cervical dilatation on admission and blood test results. Group 3 showed a longer time until delivery compared with that in group 1. There was no significant difference between the amounts of intrapartum hemorrhage, but the amount in group 3 was slightly less. All patients in group 1 and group 2, and half of the patients in group 3 received a blood transfusion. None of the patients in group 1 and one case each in group 2 and group 3 developed disseminated intravascular coagulation (DIC) before delivery. The period of hospitalization was 12±8 days in group1, 14±7 days in group 2, and 7±2 days in group 3.

Conclusion: We could not demonstrate whether vaginal delivery or cesarean section was better in placental abruption with fetal death in this study. However, this study suggested that patients with vaginal delivery had less intrapartum hemorrhage and lower frequency of blood transfusion rather than patients undergoing cesarean section.

Key words; placental abruption, fetal death, vaginal delivery, cesarean section

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緒　言

常位胎盤早期剥離（早剥）は、正常位置に付着している胎盤が、妊娠中または分娩経過中の胎児娩出以前に子宮壁より剥離するものと定義されている。[1)]

早剥は出血性ショックやDICを合併しやすく、胎児死亡や妊産婦死亡の原因となる。日本産科婦人科学会がまとめた周産期統計によると、2001年から2004年の妊産婦死亡28例のうち、前置胎盤ないし胎盤早期剥離が原因であったのは3例（10.7%）であり、2005年の周産期死亡930例のうち、早剥が原因であったのは87例（9.4%）であった。[2)]

胎児が生存している場合は急速遂娩が基本であるが、早剥により胎児が既に死亡している場合、ふたつの異なる管理方針がある。ひとつはDICの進行を防止するため死亡胎児や剥離した胎盤の早期娩出が重要であるとの観点から、すみやかに帝王切開術を行う方針である。もうひとつは分娩を急がず、まず十分な輸液、輸血あるいは凝固因子の補充を行い、全身状態を安定させた後に積極的に経腟分娩を試みる方針である。いずれの方針をとるか症例毎に検討されているが、明確な判断の基準があるわけではない。母体の全身状態を安定化させた後の経腟分娩方針と急速遂娩方針を比較して母体予後に差がなく、分娩までの時間が長くなっても母体予後には影響がないという報告から、胎児死亡を伴う早剥の場合、出血が大量で外科的処置が必要と判断された例や凝固障害が悪化し続ける例以外は、人工破膜やオキシトシンを併用した積極的な経腟分娩が推奨されている[3~5)]。産婦人科診療ガイドラインでは、施設のDIC対応能力や患者の状態により、経腟分娩促進、あるいは緊急帝王切開のいずれかの方針を採用するとされている。[6)]

今回、子宮内胎児死亡を伴う早剥例を、ただちに帝王切開術を行った例、経腟分娩を行った例、およびはじめ経腟分娩を試みたが後に帝王切開術に変更した例に分け、母体の転帰を後方視的に比較検討した。

方　法

1994年1月から2008年3月（15年間）の当院受診例のうち、分娩前に早剥による子宮内胎児死亡と診断された19例を対象とした。うち16例は他院からの救急搬送例であった。分娩前の早剥の診断は性器出血や下腹痛などの自覚症状および超音波検査によりなされ、分娩時の胎盤の肉眼所見や病理学的所見により確認した。当科では胎児死亡例は基本的に経腟分娩を選択する方針としているが、最終的な判断は母体の状態などにより症例毎に担当医が決定した。

ただちに帝王切開術を行った例（帝切群）、はじめ経腟分娩を試みたが後に帝王切開術に変更した例（変更群）、および経腟分娩を完遂した例（経腟群）、以上3群について母体年齢、分娩週数、分娩歴、妊娠合併症、入院時の内診所見、分娩所要時間、分娩時出血量と輸血、血液検査所見、血液凝固検査所見、および入院期間について検討した。推計学的検討はスチューデントのt検定あるいはクラスカル・ワーリス検定によった。

結　果

胎児死亡を伴う早剥19例のうち帝切群は5例（26%）、変更群は4例（21%）、経腟群は10例（53%）であった。

母体年齢（才）は帝切群 32±2.7（平均±標準偏差、以下同じ）、変更群 32±6.5、経腟群 33±5.2 および分娩週数（週）帝切群 34±4、変更群 34±1、経腟群 34±5 は3群間に差がなかった。経腟群には経産婦が多い傾向があった（帝切群3/5例（60%）、変更群1/4例（25%）、経腟群8/10例（80%））。早剥の危険因子である妊娠高血圧症候群あるいは子宮内胎児発育遅延あるいは喫煙の合併率（%）は、帝切群2/5例（40%）、変更群2/4例（50%）、経腟群5/10例（50%）であり3群間に差がなかった（Table 1）。

入院時の頸管開大度（cm）は帝切群2±1、変更群3±1、経腟群2±1であり、3群間に差がなかった。経腟群の7/10例（70%）および変更群の2/4例（50%）では人工破膜、機械的頸管拡張あるいはオキシトシン点滴静注のいずれか、または併用による陣痛誘発ないし促進を行った。診断から分娩までの時間（分）は帝切群116±39、変更群253±281、経腟群342±149で、経腟群は帝切群と比較して約3倍時間がかかっていた（Table 2）。

入院時のヘモグロビン値（g/dl）は3群間に差がなかった（帝切群10.1±0.8、変更群9.5±2.7、経腟

群9.5±1.4)。血小板≦10万/μlの例は帝切群にはなかった（帝切群0/5例、変更群2/4例（50%)、経腟群3/10例（30%))。フィブリノーゲン≦150mg/dlの例の割合（帝切群2/5例（40%)、変更群1/4例（25%)、経腟群5/10例（50%))、プロトロンビン時間≦50%の例の割合（帝切群0/5例、変更群0/4例、経腟群3/10例（30%)）およびFDP≧10μg/mlの例の割合（帝切群3/5例（60%)、変更群2/4例（50%)、経腟群7/10例（70%))は3群間で差がなかった（Table 3)。

Table 1. Matenal background

	Group 1 (n=5)	Group 2 (n=4)	Group 3 (n=10)
Maternal age (years)	32±2.7	32±6.5	33±5.2
Gestational age at delivery (weeks)	34±4	34±1	34±5
pluripara	3 (60%)	1 (25%)	8 (80%)
Risk factor	2 (40%)	2 (50%)	5 (50%)
PIH	2	1	1
IUGR	0	1	1
smoking	0	0	2

PIH： Pregnancy induced hypertension, IUGR： Intrauterine growth restriction (mean±SD)
Group 1: cesarean section, Group 2: cesarean section after attempting vaginal delivery, Group 3: vaginal delivery

Table 2. Cervical dilatation、induction of labor、time until delivery

	Group 1 (n=5)	Group 2 (n=4)	Group 3 (n=10)
cervical dilatation (cm)	2±1	3±1	2±1
induction of labor	0	2 (50%)	7 (70%)
time until delivery (min)	116±39	253±281	342±149

(mean±SD)

Table 3. Blood test results

		Group 1 (n=5)	Group 2 (n=4)	Group 3 (n=10)
Hb (g/dl)		10.1±0.8	9.5±2.7	9.5±1.4
Plt≦100,000/μl	On admission	0	2 (50%)	3 (30%)
	After delivery	3 (60%)	1 (25%)	5 (50%)
Fib≦150mg/dl		2 (40%)	1 (25%)	5 (50%)
PT≦50%		0	0	3 (30%)
FDP≧10μg/ml		3 (60%)	2 (50%)	7 (70%)

Hb: hemoglobin, Plt: platelet, Fib: fibrinogen, PT: prothrombin time, FDP: fibrin degradation products (mean±SD)

Table 4. The amounts of intrapartum hemorrhage、blood transfusion、disseminated intravascular coagulation (DIC) score

	Group 1 (n=5)	Group 2 (n=4)	Group 3 (n=10)
The amounts of intrapartum hemorrhage (ml)	2,089±797	1,581±327	1,682±1537
blood transfusion	5 (100%)	4 (100%)	5 (50%)
blood transfusion after delivery	5	2	1
DIC score≧8 points	0	1 (25%)	1 (10%)

(mean±SD)

分娩時出血量（ml）は帝切群2,089±797、変更群1,581±327 、経腟群1,682±1,537で、 3 群間で有意な差を認めなかったが、帝切群でやや多い傾向にあった。輸血を要した例は帝切群＋変更群では全例（9 / 9 例)、経腟群では 5 /10例（50%）であった。経腟群の 4 / 5 例および変更群の 2 / 4 例は分娩前、帝切群の 5 / 5 例は分娩後に輸血を開始した。分娩前に産科DICスコア（真木ら）が 8 点以上であったのは、帝切群 0 / 5 例、変更群 1 / 4 例 （25%)、経腟群 1 /10例（10%）であった（Table 4)。

分娩から退院までの入院期間（日）は帝切群12±8、変更群14± 7 、経腟群 7 ± 2 であった。

帝王切開術を行った理由は、帝切群ではDICの進行（1 / 5 例）および横位（1 / 5 例)、本人の希望（1 / 5 例)、不明（2 / 5 例)、変更群では分娩停止（3 / 4 例）および急性腎不全（1 / 4 例）であった。帝切群、変更群、経腟群いずれにおいても、子宮出血のために子宮全摘術を要した例はなかった。

考 察

今回胎児死亡を伴う早剥19例を検討し、最終的に10例が経腟的に、 9 例が帝王切開術で分娩した。分娩様式は母体の状態により主治医が判断したが、分娩様式を選択する基準は明確ではない。来院時の頸管の開大度やヘモグロビン値、血液凝固系検査結果、DICスコアの値などは経腟群、帝切群あるいは変更群の間で違いはみられなかったが、血小板数が10万以下の例および経産婦では経腟分娩が多く選択されていた。血小板が減少していると、術中の出血コントロールが困難になると判断された可能性がある。また、経産婦では分娩が進行しやすいため、経腟分娩が選択されたと考えられる。

Williams obstetrics 22版には、死亡した胎児をいつまでに分娩させればよいかというエビデンスはないが、分娩までにかかった時間より、適切な輸液・輸血が行われたかどうかが母体予後に影響するという報告が記載されている[7]。このことから、欧米では出血が大量で外科的処置が必要と判断された例や凝固障害が悪化し続ける例以外は、積極的な経腟分娩が推奨されている[5]。

わが国での検討で、野田らは[8]、経腟分娩した15例と帝王切開術を行った 7 例を比較し、経腟分娩で母体予後が良好であったと報告している。

当然ながら経腟分娩では帝王切開術と比較して分娩時間が長い。したがって子宮内に残存する死亡胎児や剥離した胎盤がDICを増悪させるおそれがある。今回の検討では分娩までにかかった時間は経腟群は帝切群の約 3 倍であった。しかし分娩時の平均の出血量をみると帝切群と経腟群で差はなく、むしろ分娩後に輸血を要した割合は帝切群の方が高かった。分娩までに要した時間よりも輸液・輸血などの適切な補充療法が行われたか否かが母体の転帰を左右するという報告を支持する結果であった。経腟から帝切に変更された例は 4 例あったが、うち 3 例は分娩停止、1 例は急性腎不全が変更の理由であり、経腟分娩を完遂した10例と合わせ、経腟分娩の経過中に凝固障害の急激な悪化や大量出血を見た例はなかった。

今回の検討からは、胎児死亡を伴う早剥例に対し、帝王切開・経腟分娩どちらが望ましいかを判断する材料は得られなかった。しかし、分娩中にDICと判定された例を含め、多くの例で安全に経腟分娩を完

遂することが可能であり、帝王切開術を行った場合と比較して分娩時の平均の出血量は少ない傾向にあり、輸血を要する割合も低いことが示唆された。

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Severe preterm IUGRにおけるconfined placental mosaicismの関与
—特に周産期から出生後12カ月について—

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目的

原因不明のsevere preterm IUGR（以下，IUGR）には，しばしばconfined placental mosaicism（CPM）が認められる[1]。CPMとは，胎児は正常核型であるにもかかわらず胎盤にのみ染色体異常を認める病態であり，直接的には染色体異常に伴う胎盤機能不全，間接的には胎児のuniparental disomy（UPD）あるいはインプリンティング異常のためIUGRをきたす可能性が指摘されている（図1）[2,3]。

私どもは，原因不明のFGR（IUGR）における周産期事象や出生後の発育・発達とCPMとの関連について検討した。

対象・方法

本研究は倫理委員会の承認を得て，説明と同意のもとに行われた。

1 対象

1996年から2007年に当科で分娩した3,257例のうち，多胎妊娠，染色体異常，感染，妊娠高血圧症候群，糖尿病，喫煙，アルコール，臍帯因子を有する例はIUGRのリスク因子として除外した。妊娠週数は妊娠初期の超音波検査で確認し，原因不明のFGR（IUGR）（＜－2SD）50例および患者背景をマッチさせた正常妊娠50例を対象とした。それぞれ臍帯血，胎盤組織，および両親の末梢血を一組として採取した。

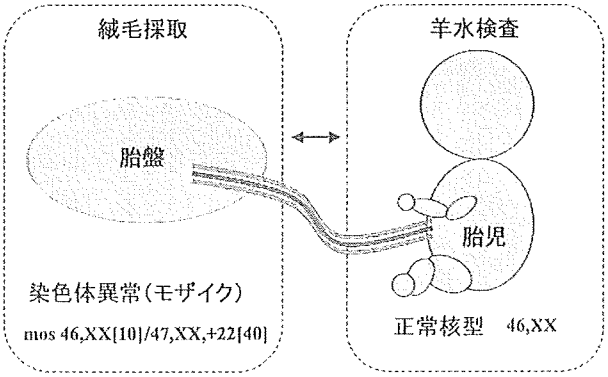
2 細胞遺伝学的検査

胎盤組織を胎盤の2カ所以上の部位から採取し，50～100個の培養細胞についてGバンド法で染色体検査を行った。そして，2つのフラスコにおけるモザイクの染色体核型が一致したものをモザイクと判定した。臍帯血についても同様に染色体検査を行った。胎盤組織の染色体核型がモザイクで，臍帯血のそれが正常核型のものをCPMと判定した。

3 分子遺伝学的検査

CPMと判定された例について，それぞれ臍帯血，胎盤組織，および両親の末梢血よりキットを用いてDNAを抽出した。DNA多型マーカー（ABI PRISM linkage-mapping set-MD10，Applied Biosystems）を用いて，遺伝子型解

図1　Confined placental mosaicism（CPM）



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