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Letter to the Editor

Whole exome sequencing revealed biallelic *IFT122* mutations in a family with CED1 and recurrent pregnancy loss

To the Editor:

Cranioectodermal dysplasia-1 (CED1), also known as Sensenbrenner syndrome (MIM 218330), is characterized by skeletal, craniofacial, and ectodermal abnormalities (1). Here, we report a family with CED1 and recurrent abortions.

I-2, 39-year-old woman, was referred to our hospital for consultation regarding recurrent abortions. Although she had one healthy boy (II-2), she suffered from two artificial abortions due to fetal hydrops at 13 weeks of gestation (II-1) and skeletal anomalies at 21 weeks of gestation (II-8), one intrauterine fetal death with hydrops at 13 weeks of gestation (II-7), and four recurrent miscarriages (II-3 at 6 weeks, II-4 at 8 weeks, II-5 at 8 weeks, and II-6 at 7 weeks) (Fig. 1a). Postmortem physical findings of II-8 included a skull deformity and blisters beside the nasal bridge due to obstetric intervention, low set ears, nuchal edema, a narrow thorax, and acromelic shortening of the limbs, posterior bowing of the lower legs and bilateral 2–3 toe syndactyly (Fig. 1b). Postmortem radiography and 3-D computed tomography showed generalized skeletal alterations which were thought to fit to CED1, though the sharp angulation of the tibiae was very unusual (Fig. 1b).

Exome sequencing was performed in I-2, II-2, and II-8 as previously described (2). We identified compound heterozygous mutations in *IFT122* in the fetal skeletal anomalies (II-8): c.1108delG (p.E370Sfs*51) in exon 11 and c.1636G>A (p.G546R) in exon 14 was inherited from his mother (Fig. 1c,d). Of note, we confirmed the same compound heterozygous mutations (c.1108delG: 2 of 29 clones, c.1636G>A: 13 of 30 clones) by capillary sequence of polymerase chain reaction (PCR) product from cloned DNA from paraffin-embedded chorionic villi (II-6) (Fig. 1e). Accordingly, c.1108delG found in II-6 and II-8 was presumed to be inherited from the father, though it was not directly confirmed.

Walczak-Sztulpa et al. reported homozygous missense mutations of *IFT122* in three patients from two consanguineous pedigrees with CED1: p.V553G in family CED-01, p.S373F in family CED-02, and compound heterozygous mutations, c.502+5G>A and p.W7C in one sporadic case, family CED-03 (1) (Fig. 1c). The four patients with *IFT122* mutations showed skeletal anomalies. We found compound heterozygous mutations in a fetus with skeletal anomalies (II-8) and the villous tissues (II-6). Clinical skeletal features of the aborted fetus (II-8) are consistent with those of the reported CED patients carrying biallelic *IFT122* mutations. Because they were found in the villous tissues (II-6) that was sequenced, biallelic *IFT122* mutations may have caused some of the recurrent abortions in this family. All four *IFT122* missense mutations (including ours) are predicted to be damaging for IFT122 function (Table 1).

CED1 belongs to a group of short rib dysplasias, including Ellis van Creveld syndrome, Jeune asphyxiating thoracic dysplasia, short rib polydactyly syndrome (SRPS) I (Saldino-Noonan), SRPS II (Majewski), SRPS III (Verma-Naumoff) and SRPS IV (Beemer-Langer) (3). The skeletal manifestation of the present fetus shared some features with other short rib dysplasias. For example, humeral bowing resembles that commonly seen in Ellis van Creveld syndrome. Severe tibial angulation was somewhat reminiscent of tibial hypoplasia in SRPS II (Majewski type). Generally, CED1 is a non-lethal disorder. However, the present family showed recurrent abortions, which can be considered as the severest phenotypes caused by biallelic *IFT122* mutations in human. Interestingly, *Ift122*-null mice show multiple developmental defects with embryonic lethality consistent with recurrent pregnancy loss in our family (4).

On the basis of the assumption that recessive mutations may cause recurrent pregnancy loss, the literatures have been carefully reviewed, but only a homozygous *HERG* mutation in a family were found to show recurrent intrauterine fetal loss (5).

In conclusion, we were able to find causative *IFT122* mutations in a non-consanguineous family with recurrent abortions. This information is useful for future counseling including preimplantatory diagnosis in this family.

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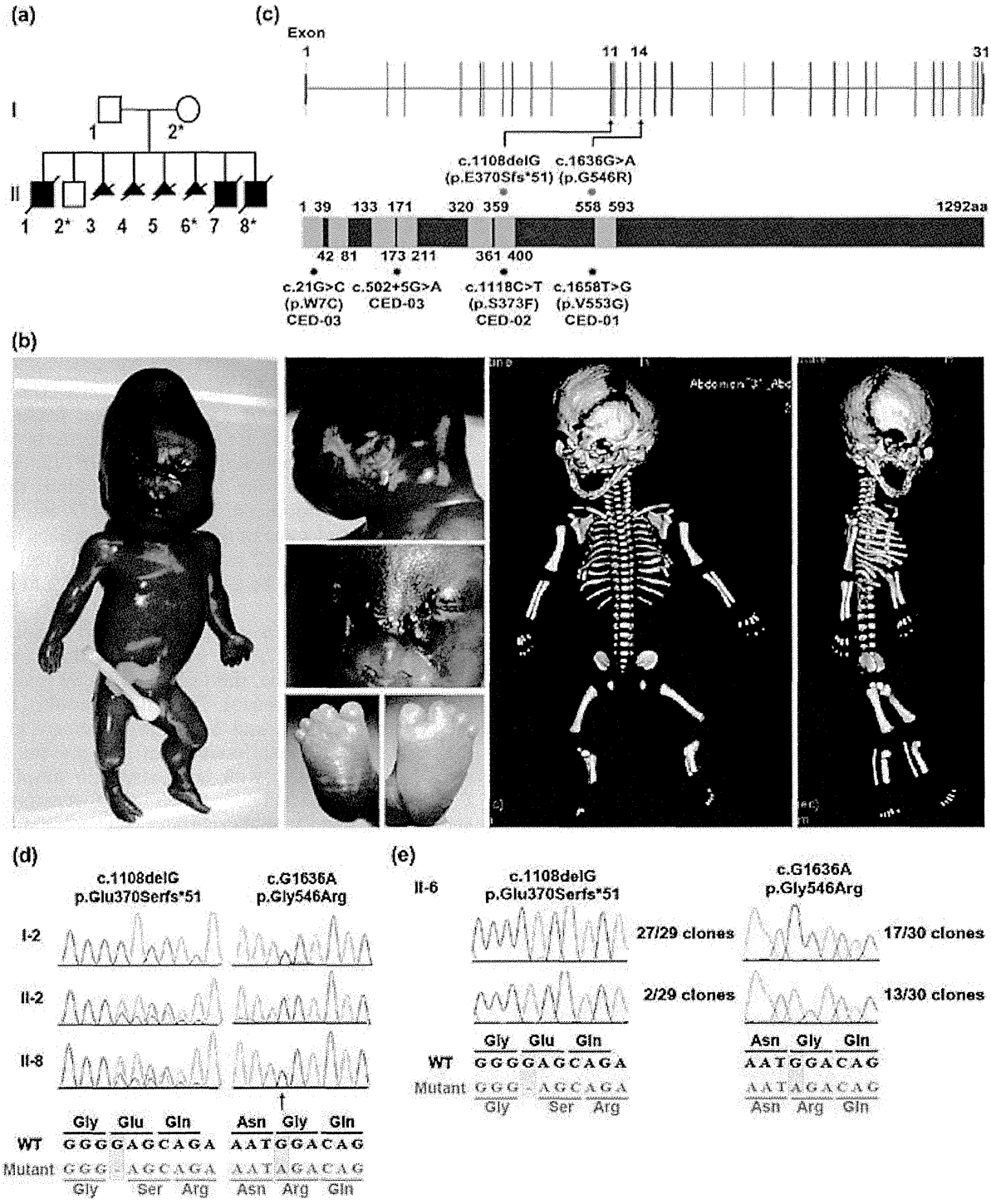

Fig. 1. Clinical finding of fetal skeletal anomalies (II-8) and genetic studies. (**a**) Familial pedigree. II-1 and II-8 were artificially terminated, II-3, II-4, II-5, II-6 and II-7 were spontaneously aborted. * DNA was available. (**b**) Clinical photographs and 3D computed tomography of II-8. (i–v) Postmortem physical findings included a skull deformity due to obstetric intervention, blisters beside the nasal bridge, low set ears, nuchal edema, a narrow thorax, and acromelic shortening of the limbs, anterior bowing of the lower legs and bilateral 2–3 toe syndactyly. (vi–vii) Postmortem 3-D computed tomography showed generalized skeletal alterations. The thorax was narrow with short ribs. The lower ribs showed a wavy appearance. The spine and ilia were normal. The long bones were not apparently short. However, the humeri were medially bowed, and the tibiae showed sharp bending at their proximal part. Ossification of the proximal and middle phalanges was defective. (**c**) The gene structure of *IFT122* (upper) and its protein structure (lower) containing seven WD40 domains (blue). Mutations found in this family and in the previous report are depicted above and below the protein, respectively. (**d**) Sequence electropherogram of family members. Compound heterozygous mutations are indicated in II-8, while healthy members (I-2 and II-2) only carry one of the two mutations. (**e**) Sequence electropherogram of the villous tissue at 7 weeks of gestation (II-6). Amplified PCR products were cloned into pCR4-TOPO vector and each clone was subjected to sequencing. Compound heterozygous mutations are indicated.

Table 1. *IFT122* mutation and their pathogenicity prediction

	Allele 1					Allele 2				
Family; Ref.	Nucleotide change	Amino acid change	SIFT	PolyPhen-2	MutationTaster	Nucleotide change	Amino acid change	SIFT	PolyPhen-2	MutationTaster
CED-01; Walczak-Sztulpa et al. (1)	c.1658T>G	p.V553G	0.00; damaging	1.000; probably damaging	1.000; disease causing	c.1658T>G	p.V553G	0.00; damaging	1.000; probably damaging	1.000; disease causing
CED-02; Walczak-Sztulpa et al. (1)	c.1118C>T	p.S373F	0.00; damaging	1.000; probably damaging	1.000; disease causing	c.1118C>T	p.S373F	0.00; damaging	1.000; probably damaging	1.000; disease causing
CED-03; Walczak-Sztulpa et al. (1)	c.21G>C	p.W7C	0.00; damaging	1.000; probably damaging	1.000; disease causing	c.502+5G>A	Splice-site change	–	–	–
This study	c.1636G>A	p.G546R	0.00; damaging	1.000; probably damaging	1.000; disease causing	c.1108delG	p.E370Sfs*51	–	–	–

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Multiple pregnancy, short cervix, part-time worker, steroid use, low educational level and male fetus are risk factors for preterm birth in Japan: A multicenter, prospective study

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Abstract

Aim: To examine the relationship between preterm birth and socioeconomic factors, past history, cervical length, cervical interleukin-8, bacterial vaginosis, underlying diseases, use of medication, employment status, sex of the fetus and multiple pregnancy.

Methods: In a multicenter, prospective, observational study, 1810 Japanese women registering their future delivery were enrolled at 8^{+0} to 12^{+6} weeks of gestation. Data on cervical length and delivery were obtained from 1365 pregnant women. Multivariate logistic regression analysis was performed.

Results: Short cervical length, steroid use, multiple pregnancy and male fetus were risk factors for preterm birth before 34 weeks of gestation. Multiple pregnancy, low educational level, short cervical length and part-timer were risk factors for preterm birth before 37 weeks of gestation.

Conclusion: Multiple pregnancy and cervical shortening at 20–24 weeks of gestation was a stronger risk factor for preterm birth. Any pregnant woman being part-time employee or low educational level, having a male fetus and requiring steroid treatment should be watched for the development of preterm birth.

Key words: cervical length, fetal sex, multiple pregnancy, part-time worker, steroid use.

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Introduction

Preterm birth (PTB), defined as delivery before 37 weeks of gestation, is a major cause of neonatal morbidity and mortality worldwide.[1,2] The Ministry of Health, Labor and Welfare in Japan reported that the incidence of PTB has increased in the last two decades. In 2010, preterm newborn infants represented 5.7% of live births. Several risk factors for PTB have been reported in many countries.[3–14] However, genetic background, environmental factors, and medical systems or quality could affect the risk differently in each country.

Clarifying the natural history of PTB and its associated risk factors in Japanese women will guide maternal–infant health-care professionals in applying a preventive strategy to reduce PTB. However, there has been no multicenter, prospective, observational study undertaken in Japan to clarify the risk factors for PTB. The objective of this study is to examine the prevalence of spontaneous PTB and determine the risk factors for PTB.

Methods

Study design and participants

A multicenter study was implemented in 14 reference obstetric units throughout Japan under the coordination of the Department of Obstetrics and Gynecology, University of Toyama, Toyama, Japan. The study was conducted from April 2008 to March 2010 and was approved by the institutional review board of each participating hospital or center. Participants provided written informed consent to study coordinators or investigators prior to participation in this study.

Women between 8^{+0} and 12^{+6} weeks of gestation were eligible for this study. During a regular prenatal visit, gestational age was based on the last menstrual period and the crown–rump length (CRL) around 9 weeks of gestation using transvaginal ultrasonography. If not, the CRL at approximately 9 weeks or the biparietal diameter after 12 weeks was used to define gestational age.

Data collection included maternal age, family income per year, educational level, previous pregnancy history (spontaneous miscarriage, induced abortion, spontaneous PTB, induced PTB), medical complications, underlying disease, smoking and alcohol intake habits, occupational status (full-time worker, part-time worker, full-time housewife), usage of medication (steroid for collagen disease or bronchial asthma, antihypertensive), Gram staining of cervical mucus, cervicovaginal interleukin-8 (IL-8), cervical length by vaginal ultrasonography at 20–24 weeks and sex of the fetus (Table 1).

Table 1 Risk factors for preterm birth examined in this study

Education
Family income
Obstetric history (e.g. spontaneous abortion, spontaneous preterm birth, stillbirth, fetal growth restriction, pregnancy-induced hypertension)
Infections (bacterial vaginosis, *Chlamydia trachomatis*, *Candida*)
Cigarette smoking and alcohol intake before and during pregnancy
Medications (steroids only for collagen disease or bronchial asthma, antihypertensives, anti-asthmatics and antidepressants)
Employment status (housewives, full-time worker, part-time worker)
Medical history or underlying disease (central nervous system, asthma, renal disease, heart disease, thyroid disease, bone or muscle disease, uterine myoma, collagen disease, hypertension, diabetes mellitus, psychological disease)
Gram staining at 8–12 weeks (modified Nugent score by Verstraeren)
Cervical length at 20–24 weeks
Cervical interleukin-8
Complication and pregnancy outcome in this pregnancy
Sex of fetus (male : female)

The first part of this study was scheduled between 8^{+0} and 12^{+6} weeks of gestation, and the second part was between 20 and 24 weeks of gestation. Cervical cytology and IL-8 samplings were carried out in each part, whereas the measurement of cervical length was performed only in visits between 20 and 24 weeks. The samples for Gram staining and IL-8 were obtained from cervical discharge with a small cotton swab applied for 10 s before the performance of any other portion of the pelvic examination. The samples for Gram staining were transferred to the Mitsubishi Chemical Medience Corporation (Tokyo, Japan) and were classified by the method of Verstraelen *et al.*[15] The samples for IL-8 were placed in buffer, frozen at–80°C within 2 h of collection, transferred to the University of Toyama, and analyzed within a few weeks using the enzyme-linked immunoassay method.[16] The level of cervicovaginal IL-8 and the category after modified classification of Gram staining were not announced to 14 reference obstetric units. Bacterial vaginosis (BV) was diagnosed and treated at respective hospitals.

The cervical length was measured with a transvaginal real-time ultrasonographic probe with an empty

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maternal bladder. The appropriate view for measurement was determined by finding a faint line of echodensity or echolucency between the external os and the internal os. Undue pressure on the cervical canal, which might have artificially created the impression of a longer cervix, was avoided by withdrawing the probe until the image blurred and then reapplying only sufficient pressure to restore the image. The cervix was measured along the line made by the interface or the mucosal surfaces, and calipers were placed at the notches made by the internal os and the external os. The cervical length measurement recorded was the shortest measurement that was sufficiently clearly displayed.[17–20]

From all the participating centers, 1810 women were initially enrolled in the study (Fig. 1). Because we could not collect detailed data on pregnancy outcome from 218 women and on cervical length from 227 women, we recruited a total of 1365 women between April 2008 and March 2010.

The distribution of enrolled women by study center was as follows: Juntendo Urayasu Hospital (23.5%, $n = 321$), Aiiku Hospital (21.0%, $n = 286$), Sendai Medical Center (11.2%, $n = 153$), Hirosaki National Hospital (10.8%, $n = 147$), Okayama Medical Center (9.5%, $n = 129$), Seichokai Fuchu Hospital (7.3%, $n = 100$), Toyama University Hospital (6.2%, $n = 85$), Kyushu Medical Center (4.2%, $n = 57$), Kure Medical Center (3.4%, $n = 46$), Hamamatsu University Hospital (2.2%, $n = 30$) and National Center for Global Health and Medicine (0.8%, $n = 11$).

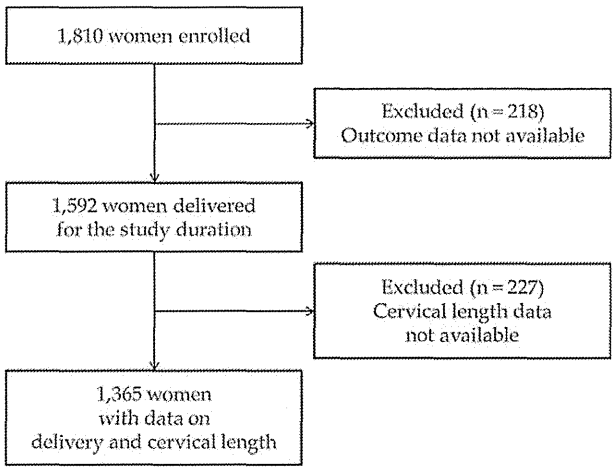

Figure 1 Participant flow diagram.

Statistical analysis

After univariate analysis, multivariate logistic regression analyses were employed to identify independent predictors of PTB. Odds ratios (OR) and 95% confidence intervals (95% CI) were reported with two-tailed probability (P) values. Statistical calculations were carried out using SAS ver. 17.1. A two-tailed P-value of 0.05 was used to define statistically significant results.

Results

Demographic and clinical characteristics

The majority of participants (99.5%) had completed high school. Approximately half of the women (49.8%) reported a high family income (≥¥5 million/year), 32.7% an intermediate income (¥2.00–4.99/year) and 1.9% a low income (<¥2 million/year). Twenty-four percent of women had experienced spontaneous abortion before 12 weeks. Although 30.0% of women quit smoking after their pregnancy was identified, 3.1% of women never quit smoking. Forty-two percent of women were housewives, while 14% of them worked part-time.

From the 1365 women, 42.3% presented with grade I microflora at 8–12 weeks, 1.4% showed an episode of grade I-like microflora, 23.1% showed an episode of heavy vaginal leukocytosis (grade I polymorphonuclear leukocytes [PMN]), while 21.3% of women had BV-like microflora.

The median maternal age was 32 years and the mean gestational age at delivery was 39 weeks. One hundred and two (7.5%) women experienced a spontaneous PTB before 37 weeks, while 19 (1.4%) did so before 34 weeks.

We have had six patients with steroid use (four patients for collagen disease, one patient for renal disease and one patient for bronchial asthma) and we included these six patients in multivariate logistic analysis. Two of six patients delivered preterm babies. To restrict the steroid users to the patients who had collagen disease or bronchial asthma, we counted the patients who had steroid for fetal lung maturity as non-steroid users.

Risk factors for PTB before 34 weeks of gestation

Table 2 provides details of the univariate analysis and the multivariate logistic regression analysis of risk factors for PTB before 34 weeks of gestation. In univariate analysis, low educational level, history of miscarriage or PTB, threatened abortion (atypical genital

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Table 2 Risk factors for preterm birth before 34 weeks of gestation

		Univariate analysis			Multivariate analysis		
		OR	95% CI	*P*	OR	95% CI	*P*
Educational level							
1	Junior high school	26.29	2.60–265.75	0.0000**	10.20	0.50–208.09	0.13
2	Others						
Family income							
1	<¥2 million/year	3.1	0.40–24.24	0.26	—	—	—
2	Others						
Employment							
1	Part-time	1.23	0.35–4.23	0.75	—	—	—
2	Others						
History of miscarriage or preterm birth							
1	Yes	3.01	1.18–7.69	0.01547*	2.72	0.93–7.94	0.07
2	No						
History of cervical surgery (cone biopsy)							
1	Yes	N/A	N/A	N/A	—	—	—
2	No						
Uterine myoma							
1	Yes	2.54	0.57–11.30	0.20	—	—	—
2	No						
Threatened abortion							
1	Yes	3.67	1.19–11.37	0.0158*	1.75	0.44–7.02	0.43
2	No						
Subchorionic hematoma							
1	Yes	2.87	0.37–22.34	0.29	—	—	—
2	No						
Cervical length at 20–24 weeks <25 mm							
1	Yes	34.87	11.85–102.63	0.0000**	0.89†	0.84–0.94†	0.0000**
2	No						
Steroid use for collagen disease or bronchial asthma							
1	Yes	15.75	1.75–142.13	0.0097**	31.94	2.04–500.26	0.0136*
2	No						
Multiple pregnancy							
1	Yes	11.01	3.44–35.17	0.0000**	5.53	1.34–22.79	0.0179*
2	No						
Fetal sex							
1	Male	4.85	1.40–16.84	0.006**	5.06	1.35–19.00	0.0163*
2	Female						
Body mass index <18.5							
1	Yes	0.54	0.16–1.89	0.33	—	—	—
2	No						
Smoking during pregnancy							
1	Yes	N/A	N/A	N/A	—	—	—
2	No						
Smoking before pregnancy							
1	Yes	0.46	0.13–1.61	0.22	—	—	—
2	No						
Alcohol intake during pregnancy							
1	Yes	N/A	N/A	N/A	—	—	—
2	No						
Alcohol intake before pregnancy							
1	Yes	1.19	0.47–3.00	0.72	—	—	—
2	No						
Gram staining at 8–12 weeks: grade I PMN or BV-like							
1	Yes	0.94	0.37–2.37	0.89	—	—	—
2	No						
Chlamydial infection							
1	Yes	N/A	N/A	N/A			
2	No						
Candida infection							
1	Yes	1.12	0.15–8.55	0.91			
2	No						
IL-8 level at 20–24 weeks >360 ng/mL							
1	Yes	0.87	0.27–2.80	0.82	—	—	—
2	No						

**$P < 0.01$; *$P < 0.05$. †OR (95% CI) according to mm of cervical length. BV, bacterial vaginosis; CI, confidence interval; IL, interleukin; N/A, no one had premature delivery; OR, odds ratio; PMN, polymorphonuclear leukocytes.

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bleeding at early pregnancy), steroid usage, multiple pregnancy, male fetus and short cervix at 20–24 weeks of gestation were risk factors for PTB before 34 weeks of gestation. Short cervical length, steroid use, multiple pregnancy and male fetus remained as risk factors for 34 weeks of gestation after multivariate analysis. Low family income (<¥2 million/year), part-time work, history of cervical surgery (cone biopsy), uterine myoma, subchorionic hematoma, lean women (body mass index [BMI], <18.5), cervicovaginal infection (grade I PMN or BV-like), cervical inflammation (IL-8, >360 ng/mL), smoking and alcohol intake were not risk factors for PTB before 34 weeks of pregnancy.

Risk factors for PTB before 37 weeks of gestation (Table 3)

In univariate analysis, low educational level, part-time work, uterine myoma, cervical length, steroid use for only collagen disease or bronchial asthma, and multiple pregnancy were risk factors for PTB before 37 weeks of gestation. In multivariate analysis, low educational level, part-time work, multiple pregnancy and short cervical length were risk factors for delivering a preterm baby before 37 weeks of gestation. However, family income level, uterine myoma, history of cervical surgery (cone biopsy), threatened abortion, subchorionic hematoma, fetal sex, smoking, alcohol intake, BMI of less than 18.5, high cervicovaginal level of IL-8 (>360 ng/mL) and vaginal infection in the first trimester (grade I PMN or BV-like) were not risk factors for PTB before 37 weeks of pregnancy.

Cervical length in relation to PTB

Among the 1365 women, the mean (±standard deviation) cervical length at 20–24 weeks was 42.2 ± 8.5 mm (42.5 ± 8.4 mm for nulliparous women and 38.4 ± 9.6 mm for multiparous women). The percentages of PTB before both 34 and 37 weeks of gestation increased with the progression of cervical shortening (Fig. 2). Fifty percent of women with a short cervix of less than 20 mm delivered their babies before 34 weeks of gestation. Seventy-five percent of women with cervical length of less than 20 mm gave birth before 37 weeks of gestation. The odds ratios according to millimeter of cervical length for PTB before 34 and 37 weeks are shown in Figures 3 and 4, respectively. To reveal the relationship among variables, we removed one variable which showed the largest *P*-value, and repeated this procedure until there were no variables of $P \geq 0.10$. The cervical length was the risk factors for PTB before both 34 and 37 weeks of gestation ($P = 0.0000$, odds ratio [OR] = 0.88 [95% confidence interval [CI] = 0.84–0.92]; $P = 0.0001$, OR = 0.95 [95% CI = 0.93–0.98], respectively). We also used a backward stepwise logistic regression analysis for further evaluation. The cervical length at 20–24 weeks of gestation was still the risk factors for PTB before both 34 and 37 weeks of gestation ($P = 0.0000$, OR = 0.89 [95% CI = 0.84–0.94]; $P = 0.0316$, OR = 0.97 [95% CI = 0.94–0.997], respectively).

Discussion

This is the first multicenter, prospective, observational study to detect the risk factors for PTB in Japan. Most participants in this study were women with low-risk pregnancy. Thus, the percentages of preterm delivery in this study before 32 and 37 weeks of pregnancy were 0.3% and 7.5%, respectively, which are not much higher than the levels of those delivered in Japan from 2008–2010 (0.7% and 5.7%, respectively).

The risk of PTB before 34 and 37 weeks in women with cervical length at 20 to 24 weeks of gestation decreased by approximately 11% and 5% for each additional millimeter of cervical length (OR = 0.89 [95% CI = 0.84–0.94] and OR = 0.95 [95% CI = 0.92–0.98], respectively). Werner *et al.*[21] reported that, in low-risk pregnancies, universal transvaginal cervical length ultrasonography appears to be a cost-effective strategy under a wide range of clinical circumstances. Our results support Werner's assertion.[21]

In our study, part-time work was a risk factor for PTB before 37 weeks of gestation. Part-time workers delivered preterm babies more frequently than full-time workers (13.5% vs 7.1%; $P < 0.003$). A study in the USA showed that part-time work (≤20 h/week) was reported to be associated with a lower risk of preterm labor.[6] The low levels of job stress among part-time workers may be attributable to limited exposure to physical job stress. Noborisaka *et al.*[22] showed that the levels of decision latitude in female temporary employees were lower than those in permanent employees. Kobayashi *et al.*[23] also stated that part-time employees experience more job insecurity and poorer prospects for promotion than full-timers.

In Japan, the number of part-time working women has been increasing to help with family expenses. Many pregnant part-timers are not in a situation where they can have an irregular visit without hesitation whenever they had irregular uterine contractions or they had yellowish discharge. Our data may suspect

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Table 3 Risk factors for preterm birth before 37 weeks of gestation

			Univariate analysis			Multivariate analysis		
			OR	95% CI	*P*	OR	95% CI	*P*
Educational level	1.	Junior high school	12.86	1.79–92.26	0.00105**	16.03	1.89–136.31	0.0111*
	2.	Others						
Family income	1.	Less than 2 million yen/year	1.05	0.25–4.52	0.94	—	—	—
	2.	Others						
Employment	1.	Part-timer	2.06	1.26–3.35	0.00311**	2.54	1.28–5.07	0.0080**
	2.	Others						
History of cervical surgery (cone biopsy)	1.	Yes	0.74	0.10–5.62	0.77	—	—	—
	2.	No						
Uterine myoma	1.	Yes	2.44	1.20–4.54	0.01085*	2.13	0.78–5.78	0.14
	2.	No						
Threatened abortion	1.	Yes	2.15	0.95–3.41	0.07	—	—	—
	2.	No						
Subchorionic hematoma	1.	Yes	2.15	0.73–6.32	0.15	—	—	—
	2.	No						
Cervical length at 20–24 weeks <25 mm	1.	Yes	9.24	4.03–21.15	0.0000**	0.95†	0.92–0.98†	0.0016**
	2.	No						
Steroid use for collagen disease or bronchial asthma	1.	Yes	6.42	1.16–35.48	0.01443*	8.68	0.79–94.98	0.0767
	2.	No						
Multiple pregnancy	1.	Yes	67.18	29.70–151.95	0.0000**	53.52	18.56–154.35	0.0000**
	2.	No						
Fetal sex	1.	Male	1.23	0.82–1.86	0.32	—	—	—
	2.	Female						
Body mass index <18.5	1.	Yes	0.68	0.42–1.09	0.11	—	—	—
	2.	No						
Smoking during pregnancy	1.	Yes	0.97	0.29–3.20	0.96	—	—	—
	2.	No						
Smoking before pregnancy	1.	Yes	0.9	0.57–1.42	0.66	—	—	—
	2.	No						
Alcohol intake during pregnancy	1.	Yes	0.43	0.06–3.19	0.39	—	—	—
	2.	No						
Alcohol intake before pregnancy	1.	Yes	1.1	0.73–1.65	0.65	—	—	—
	2.	No						
Gram staining at 8–12 weeks: Grade I PMN or BV-like	1.	Yes	0.96	0.64–1.44	0.85	—	—	—
	2.	No						
Chlamydial infection	1.	Yes	N/A	N/A	N/A	—	—	—
	2.	No						
Candida infection	1.	Yes	1.48	0.66–3.33	0.34	—	—	—
	2.	No						
IL-8 level at 20–24 weeks: >360 ng/mL	1.	Yes	0.48	0.25–0.92	0.02418*	0.32	0.15–0.69	0.0036**
	2.	No						

**$P < 0.01$; *$P < 0.05$. †OR (95% CI) according to mm of cervical length. BV, bacterial vaginosis; CI, confidence interval; IL, interleukin; N/A, no one had premature delivery; OR, odds ratio; PMN, polymorphonuclear leukocytes.

that some psychosocial stress in relation to part-time employment facilitate uterine contraction.

The relationship between PTB and uterine leiomyoma is based upon observational studies.[24,25] The presence of larger fibroid (>6 cm) appears to be the factor that best correlates with an increased risk of PTB.[24] Our study showed that uterine myoma was a risk factor for PTB before 37 weeks of gestation in univariate analysis, but not in multivariate analysis. This discrepancy may be due to the small number of cases ($n = 65$).

There is some evidence that women with certain autoimmune diseases, such as autoimmune thyroid disease and inflammatory bowel disease, are at

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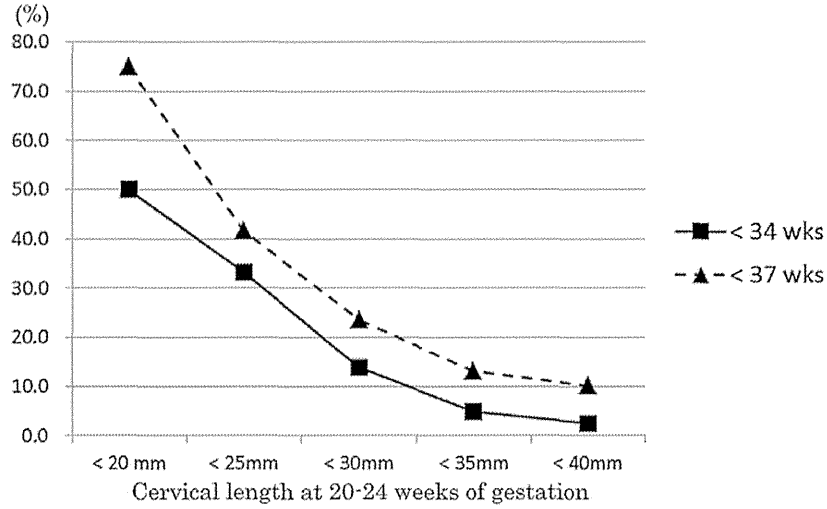

Figure 2 Observed frequency of preterm delivery before 34 weeks (solid line) and 37 weeks (dashed line) according to cervical length measured by transvaginal ultrasonography at 20–24 weeks.

Cervical length	< 20 mm	< 25mm	< 30mm	< 35mm	< 40mm
Preterm delivery (< 34wks)	6	6	10	10	12
Total delivery	12	18	72	206	479
% of preterm delivery (< 34wks)	50.0	33.3	13.9	4.9	2.5

Cervical length	< 20 mm	< 25mm	< 30mm	< 35mm	< 40mm
Preterm delivery (< 37 wks)	9	10	17	27	49
Total delivery	12	24	72	206	479
% of preterm delivery (< 37 wks)	75.0	41.7	23.6	13.1	10.2

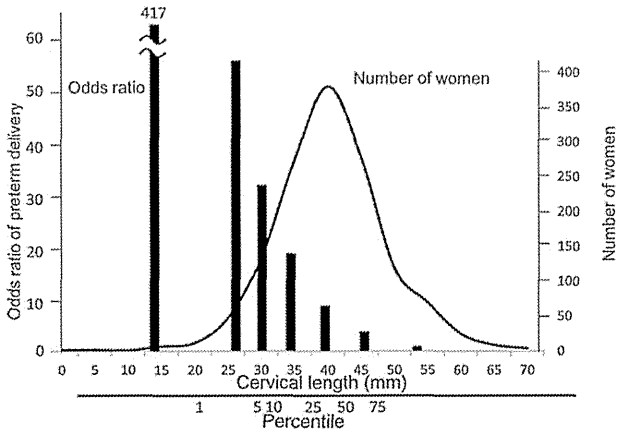

Figure 3 Distribution of subjects among percentiles for cervical length measured by ultrasonography at 20–24 weeks of gestation (solid line) and odds ratio of preterm delivery before 34 weeks of gestation according to percentiles for cervical length (bars).

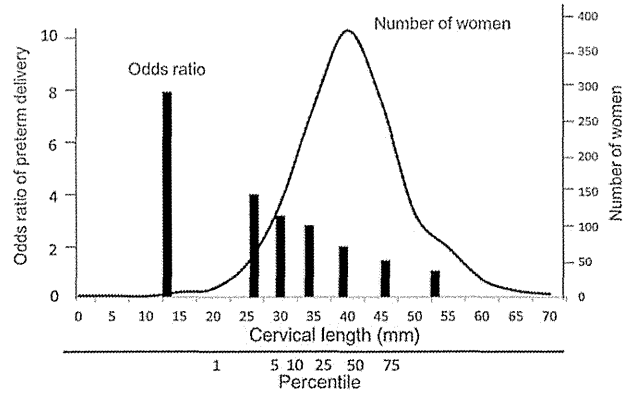

Figure 4 Distribution of subjects among percentiles for cervical length measured by ultrasonography at 20–24 weeks of gestation (solid line) and odds ratio of preterm delivery before 37 weeks of gestation according to percentiles for cervical length (bars).

increased risk for spontaneous PTB.[26,27] In our study, steroid therapy during pregnancy for asthma or collagen disease was a risk factor for PTB before 34 and 37 weeks. The association of premature labor with autoimmune function may be the consequence of abnormalities in normal fetal–placental tolerance, leading to uterine activation and labor.[28]

A number of reports have documented the relationship of a male fetus to PTB.[29–31] Yeganegi *et al.*[32] reported that lipopolysaccharide increased the output of tumor necrosis factor-α, IL-10 and prostaglandin-endoperoxide synthase-2 with a greater response in male placentae compared with female placentae and suggested that there is a greater synthesis of active prostaglandins in the placentae with male fetuses

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in a state of inflammation, which may explain the higher incidence of PTB reported for males. Our finding provides further support for the concept that women with a male fetus tend to deliver preterm babies.

Although there is some suggestion that treatment for BV before 20 weeks of gestation may reduce the risk of PTB,[33] this study's data do not suggest that women with BV-like microflora tend to deliver preterm babies. This is possibly because individual centers treated BV-positive women under the diagnosis of BV, irrespective of the result of Gram staining at 8–12 weeks in this study. Alternatively, BV may not be a risk factor for PTB in Japanese pregnant women.

There are some limitations to this study. First, the number of cases enrolled in our study was lower than expected. As a result, we could not extract many variables showing a significant difference. In future, a larger prospective study with many participating obstetricians across the country should be undertaken to identify many risk factors for PTB.

In conclusion, we must pay more attention to women who are of low educational level and part-time workers, and women who have multiple pregnancy, short cervix, steroid use for collagen disease or bronchial asthma, and male fetus to reduce the prevalence of PTB.

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