studies, with only a few reports concerning the Japanese population [17]. The aim of this study is to establish reference ranges and intra-interobserver variations for ductus venosus waveform indices of fetuses in the Japanese population, in order to facilitate the application of these indices in the prenatal fetal assessment.

Patients and methods

This is a retrospective cross-sectional study. Seven hundred ninety-one (791) singleton fetuses of healthy Japanese couples between 18 and 40 weeks of gestation were involved. They were recruited from patients visiting our routine antenatal outpatient department from January 2004 to January 2008. The study protocol was approved by the ethics committee of the institution, and informed consent was obtained for participation in this study. The criteria for participation were as follows: the gestational age was confirmed by fetal biometry (crown-rump length or biparietal diameter) between 9 and 11 weeks, and the fetuses were anatomically normal on prenatal and neonatal examinations at birth. In addition, all fetuses were term infants with an Apgar score of not less than 8 points at 1 and 5 min, their birth weights were between the 10th and 90th percentiles of the Japanese standard birth weight curve, and participants who had complications or took medications that might affect fetal circulation were excluded.

Pulsed-wave Doppler ultrasonographic examinations of fetal ductus venosus were performed transabdominally using a Voluson 730 Expert ultrasound device (GE Yokogawa Medical Systems, Tokyo, Japan) equipped with a 3.5-MHz curved-array transducer.

Using color Doppler, blood flow signals of the ductus venosus were depicted either in a mid-sagittal longitudinal plane of the fetal trunk (Fig. 1a) or in an oblique transverse plane of the fetal upper abdomen (Fig. 1b). The sample volume was positioned directly at the entrance of the ductus venosus from the umbilical vein, where color Doppler indicated the highest velocities. Low-pass filters were set at 50–100 Hz to detect all velocities appropriately. To avoid detecting signals from the adjacent hepatic and umbilical veins, the size of the sample volume was adjusted such that it only covered the entire vessel lumen. The insonation angle between the ultrasound beam and direction of blood flow was kept as low as possible and always at $\leq 50°$. All Doppler recordings used for measurements were obtained in the absence of fetal breathing movements, fetal gross body movements, and uterine contractions. Fetal heart rate was regular, within the range of 120–160 beats/min. Measurements were frequently repeated to ensure that the mechanical and thermal indices for soft tissue were maintained at or below 1.1. Only one measurement from each participant was included in the statistical analysis. Cases in which a satisfactory waveform could not be recorded were not included in the study.

The normal velocity waveform of the ductus venosus exhibits continuous, triphasic forward flow throughout the cardiac cycle. The peak forward flow velocities during ventricular systole (S); the peak forward flow velocities during early ventricular diastole (D), which corresponds to passive ventricular filling; and the lowest forward velocity or peak reversed velocity in late ventricular diastole during atrial contraction (a) (Fig. 2) were the quantities determined for ductus venosus waveform analysis. The pulsatility index (PI) was defined as (S − a)/time-averaged maximum velocity. a/S was defined as end-diastolic velocity/peak systolic velocity. PI and a/S were independent of the angle of insonation and were measured as mean values of at least three consecutive uniform waveforms. Mono- or biphasic flow patterns with comparably high late ventricular diastole were included in the analysis as they are considered a normal variant [22].

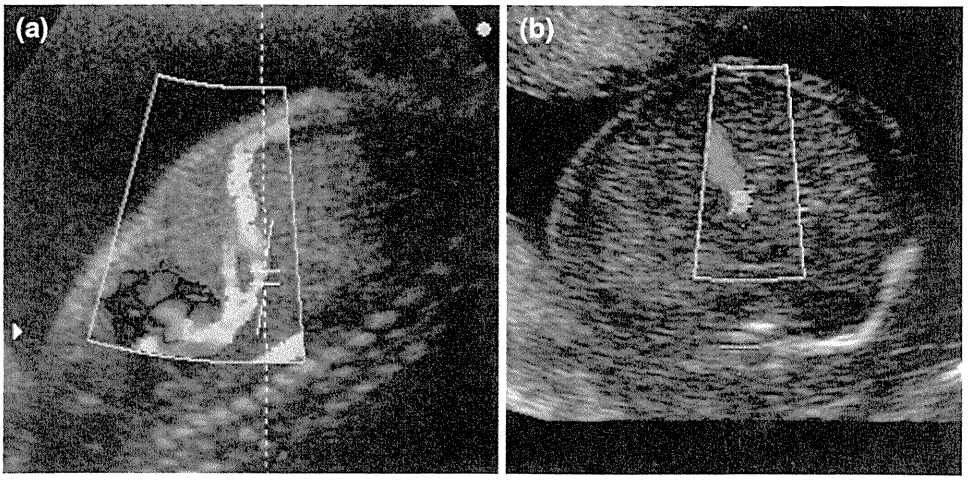

Fig. 1 Visualization of the ductus venosus **a** in a mid-sagittal longitudinal plane and **b** in an oblique transverse plane of the fetal abdomen. There is a marked difference in blood flow velocities between the umbilical vein and the ductus venosus. The higher flow velocity in the ductus venosus causes aliasing, which appears as an area of color reversal

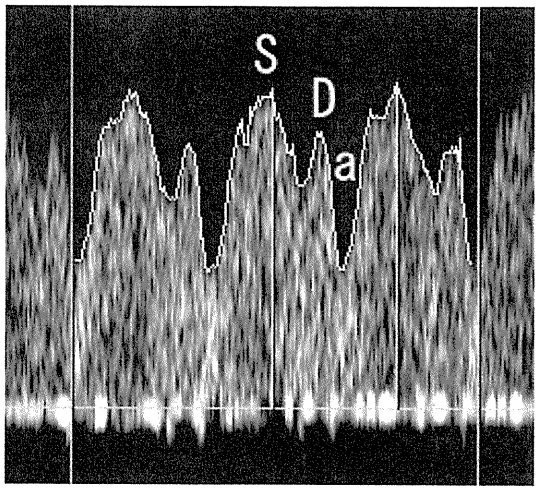

Fig. 2 Typical flow velocity waveforms of the ductus venosus in a normal fetus at 30 weeks of gestation: the peak forward flow velocities during ventricular systole (*S*); the peak forward flow velocities during early ventricular diastole (*D*), which corresponds to passive ventricular filling; and the lowest forward velocity or peak reversed velocity in late ventricular diastole during atrial contraction (*a*)

For the assessment of intraobserver variation, 52 participants were examined by one operator, and for assessment of interobserver variation, 17 participants were examined by two operators. These participants were chosen arbitrarily from all study participants and repeat Doppler measurements of the ductus venosus were performed.

Statistical analyses

The power of the study was sufficient as per previous cross-sectional studies [1, 8, 14–20]. The reference ranges for the respective gestational week were determined to construct smooth growth curves for the entire gestation period. These ranges were estimated by the methods described by Royston and Wright [23]. The assumption of normal distribution was checked for outcome variables, and Box-Cox transformation was used to achieve normal distribution. Least-squares regression analysis was performed on the transformed data to estimate both the mean and SD curves as polynomial functions of gestational age in transformed units. The 5th and 10th percentiles were calculated by subtracting 1.64 and 1.28 SD, respectively, from the mean in transformed units. The 90th and 95th percentiles were calculated by adding the respective SD multiples to the mean transformed units. The limits of the calculated reference ranges were subjected to anti-Box–Cox transformation.

Intra- and interobserver variations were calculated by the methods described by Bland and Altman [24, 25]. The mean difference was determined between these two measurements with a 95% confidence interval (CI) and limits of agreement (1.96 SD of the mean difference). Statistical analysis was performed with SPSS software (12.0.1 J for Windows; SPSS Japan, Tokyo, Japan). A P value of 0.05 was considered statistically significant.

Table 1 Background of 791 participants

Characteristic	Value
Median maternal age	32.1 years (range 19.2–45.8)
Median gestational age at delivery	39 weeks + 5 days (range 37 weeks + 0 days to 41 weeks + 6 days)
Median birth weight	3,042 g (range 2,316–3,910)
Sex of neonates	Male $n = 419$, female $n = 372$

Results

A total of 791 patients met the inclusion criteria. The background of the participants was as follows (Table 1): the median maternal age was 32.1 years (range 19.2–45.8), the median gestational age at delivery was 39 weeks + 5 days (range 37 + 0 to 41 + 6), the median birth weight was 3,042 g (range 2,316–3,910), and the fetal sex distribution was 419 males (53.0%) and 372 females (47.0%).

Satisfactory, clear, and uniform waveforms were obtained in 84% of cases (667 of 791). Fetal activity, breathing movements, and unfavorable fetal position lowered the success rate to 84%. The median PI of the ductus venosus decreased throughout the observation period, from 0.54 at 18 weeks to 0.30 at 41 weeks (Fig. 3; Table 2). The median a/S of the ductus venosus increased from 0.56 at 18 weeks to 0.76 at 41 weeks (Fig. 4; Table 3). Reference ranges for the PI and a/S of the ductus venosus with respect to each gestational age are shown in Tables 2 and 3, and regression curves of the 5th, 10th, 50th, 90th, and 95th percentiles for the same are graphically illustrated in Figs. 3 and 4. Formulae for the PI and a/S of the ductus venosus with gestational age are as follows: $\mathrm{PI} = (-0.009809 \times \mathrm{GA} + 0.7855 + K \times 0.1413)^{1.25}$ and $\mathrm{a/S} = (-0.00014 \times \mathrm{GA}^2 + 0.01512 \times \mathrm{GA} + 0.4392 + K \times 0.8787)^{10/7}$, where GA indicates gestational age (weeks) and the 5th, 10th, 50th, 90th, and 95th percentiles were calculated by $K = -1.64$, -1.28, 0, 1.28, and 1.64, respectively.

Inter- and intraobserver variations are presented as the limits of agreement (Table 4). Figures 5, 6, 7, and 8 give detailed information about inter- and intraobserver variations.

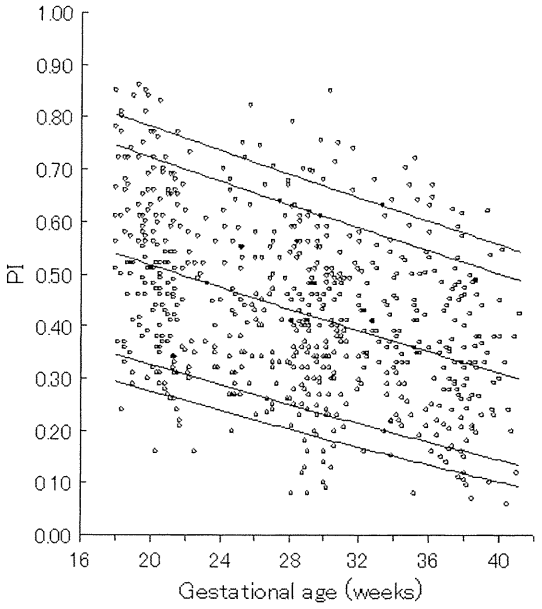

Fig. 3 Reference ranges for the pulsatility index (PI) of the ductus venosus based on 667 observations. The 5th, 10th, 50th, 90th, and 95th percentiles are shown

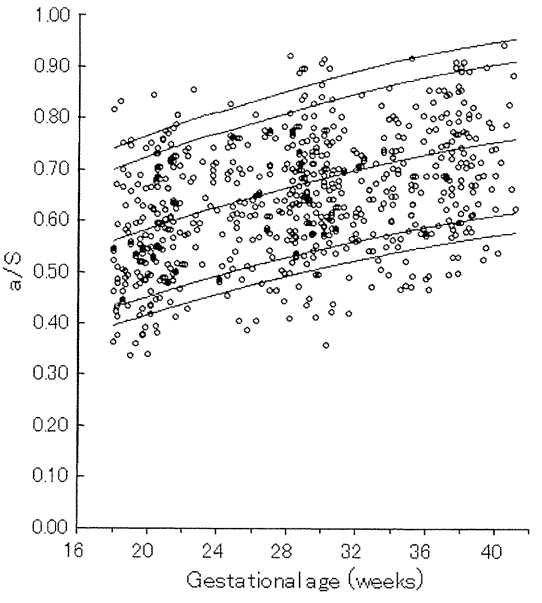

Fig. 4 Reference ranges for the end-diastolic velocity/peak systolic velocity (a/S) of the ductus venosus based on 667 observations. The 5th, 10th, 50th, 90th, and 95th percentiles are shown

Table 2 Reference ranges for the pulsatility index (PI) of the ductus venosus based on 667 observations

Gestational age (weeks)	Percentile				
	5th	10th	50th	90th	95th
18	0.30	0.35	0.54	0.74	0.80
19	0.29	0.34	0.53	0.73	0.79
20	0.28	0.33	0.52	0.72	0.78
21	0.27	0.32	0.51	0.71	0.77
22	0.26	0.31	0.49	0.70	0.76
23	0.25	0.30	0.48	0.69	0.75
24	0.24	0.29	0.47	0.68	0.74
25	0.23	0.28	0.46	0.66	0.72
26	0.22	0.27	0.45	0.65	0.71
27	0.21	0.26	0.44	0.64	0.70
28	0.20	0.25	0.43	0.63	0.69
29	0.19	0.24	0.42	0.62	0.68
30	0.19	0.23	0.41	0.61	0.67
31	0.18	0.22	0.40	0.60	0.66
32	0.17	0.21	0.39	0.59	0.64
33	0.16	0.20	0.38	0.58	0.63
34	0.15	0.20	0.37	0.56	0.62
35	0.14	0.19	0.36	0.55	0.61
36	0.13	0.18	0.35	0.54	0.60
37	0.13	0.17	0.34	0.53	0.59
38	0.12	0.16	0.33	0.52	0.58
39	0.11	0.15	0.32	0.51	0.57
40	0.10	0.14	0.31	0.50	0.56
41	0.09	0.14	0.30	0.49	0.54

Table 3 Reference ranges for the end-diastolic velocity/peak systolic velocity (a/S) of the ductus venosus based on 667 observations

Gestational age (weeks)	Percentile				
	5th	10th	50th	90th	95th
18	0.39	0.43	0.56	0.70	0.74
19	0.41	0.44	0.57	0.71	0.75
20	0.42	0.45	0.58	0.72	0.77
21	0.43	0.46	0.59	0.74	0.78
22	0.44	0.47	0.61	0.75	0.79
23	0.45	0.48	0.62	0.76	0.80
24	0.46	0.49	0.63	0.77	0.81
25	0.47	0.50	0.64	0.78	0.82
26	0.47	0.51	0.65	0.79	0.84
27	0.48	0.52	0.66	0.80	0.85
28	0.49	0.53	0.67	0.81	0.86
29	0.50	0.54	0.68	0.82	0.87
30	0.51	0.55	0.68	0.83	0.88
31	0.52	0.55	0.69	0.84	0.88
32	0.52	0.56	0.70	0.85	0.89
33	0.53	0.57	0.71	0.86	0.90
34	0.54	0.58	0.72	0.87	0.91
35	0.54	0.58	0.72	0.87	0.92
36	0.55	0.59	0.73	0.88	0.92
37	0.56	0.59	0.74	0.89	0.93
38	0.56	0.60	0.74	0.89	0.94
39	0.57	0.61	0.75	0.90	0.94
40	0.57	0.61	0.75	0.90	0.95
41	0.58	0.61	0.76	0.91	0.95

Table 4 Intra- and interobserver variation of ductus venosus flow waveform indices

	Paired differences			*df*	K-S test *P*	S-W test *P*	Limits of agreement	
	Mean (95% CI)	SD	SE				Upper (95% CI)	Lower (95% CI)
Intraobserver variation								
PI	0.00 (−0.02, 0.01)	0.06	0.01	52	>0.200	0.719	0.12 (0.09, 0.15)	−0.12 (−0.15, −0.09)
a/S	0.00 (−0.01, 0.02)	0.06	0.01	52	>0.200	0.719	0.10 (0.08, 0.12)	−0.10 (−0.12, −0.08)
Interobserver variation								
PI	0.02 (−0.01, 0.05)	0.06	0.03	17	>0.200	0.991	0.15 (0.09, 0.20)	−0.11 (−0.16, −0.05)
a/S	−0.01 (−0.03, 0.01)	0.04	0.01	17	>0.200	0.950	0.07 (0.04, 0.11)	−0.10 (−0.13, −0.06)

SD Standard deviation of the mean, *SE* standard error of the mean, *df* degrees of freedom, *K-S test* Kolmogorov-Smirnov test, *S-W test* Shapiro-Wilks test, *PI* pulsatility index, *a/S* end-diastolic velocity/peak systolic velocity

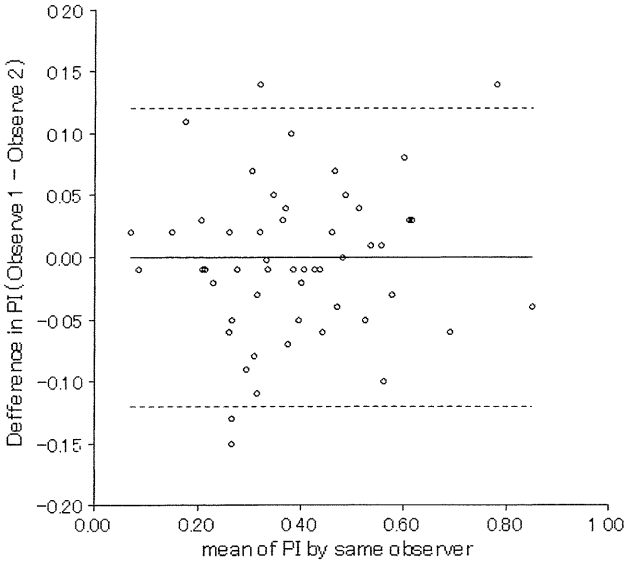

Fig. 5 Plot of difference against mean for the measurements of pulsatility index (PI) of the ductus venosus by the same observer, with the mean difference (*solid line*) and 95% limits of agreement (*dashed lines*) indicated

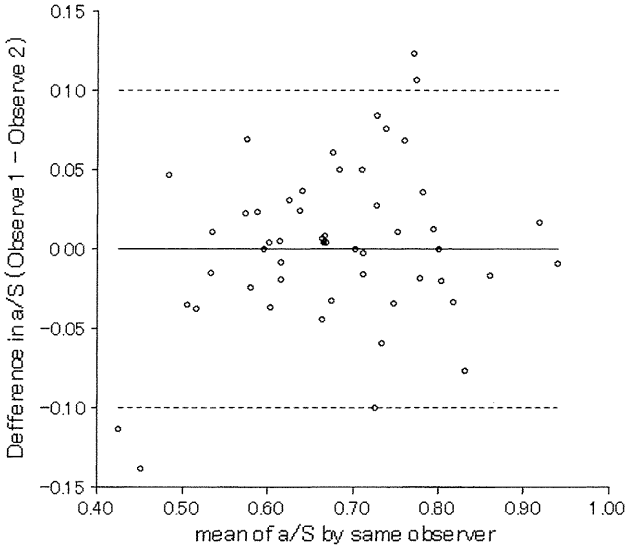

Fig. 6 Plot of difference against mean for measurements of the end-diastolic velocity/peak systolic velocity (a/S) of the ductus venosus by the same observer, with the mean difference (*solid line*) and 95% limits of agreement (*dashed lines*) indicated

Discussion

The present findings reveal that the PI of the ductus venosus decreases and the a/S of the ductus venosus increases with advancing gestation during the second and third trimesters of pregnancy. This conclusion is in agreement with those of previous studies. However, our reference ranges of PI were lower and those of a/S were higher compared to data obtained by Hecher et al. [8], Bahlmann et al. [18], Baschat et al. [19], Axt-Fliedner et al. [20], and Kessler et al. [21]. For example, Kessler et al. [21] found higher values of PI and lower values of a/S throughout the whole observation period. However, their 50th percentile value of PI was merely 0.06–0.13 (21–39 weeks) higher, and their 50th percentile value of a/S was merely 0.06–0.13 (21–39 weeks) lower compared with our data with advancing gestation. Their data were in good agreement with our data, especially at mid-gestation. These differences could have been due to variations in the study size, study population, and statistical methods. In general, the selection of the study population is extremely important when establishing reference ranges. On the one hand, Kessler et al. [21] included participants with complications that affect fetal circulation, while our approach might produce supernormal reference ranges. However, many patients were referred to our hospital for management of maternal complications or a targeted ultrasound examination for fetal anomalies or suspected IUGR, which was the reason for the exclusion of all conditions that might be associated with pathology. Reasonableness and necessity are controversial for ethnic reference ranges for fetal Doppler waveforms. However, the apparent difference from previous studies was ethnicity. Most of the reported

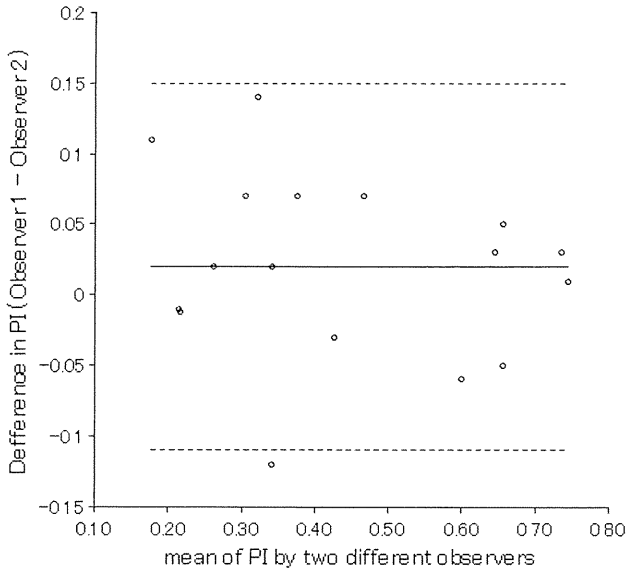

Fig. 7 Plot of difference against mean for measurements of pulsatility index (PI) of the ductus venosus by two different observers, with the mean difference (*solid line*) and 95% limits of agreement (*dashed lines*) indicated

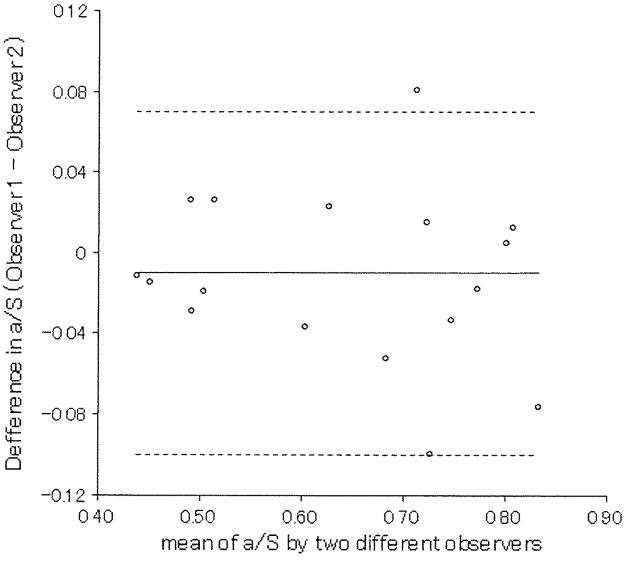

Fig. 8 Plot of difference against mean for measurements of the end-diastolic velocity/peak systolic velocity (a/S) of the ductus venosus by two different observers, with the mean difference (*solid line*) and 95% limits of agreement (*dashed lines*) indicated

data concerning reference ranges of ductus venosus waveforms have been derived from Caucasian population-based studies [1, 8, 14–16, 18–21]. There is a possibility that these reference ranges may differ in part according to ethnicity. Previously, Nakata et al. [17] reported reference ranges for flow velocities of the ductus venosus during the prenatal course in the Japanese population. However, the validity of the reference ranges is diminished by the small number of cases and the absence of information on the variation of Doppler ultrasound measurements and on the measuring parameters used.

The present inter- and intraobserver variation expressed by the 95% CI of the difference is in accordance with that reported by Kessler et al. [21] and is small enough to lead to the assumptions that any variation seen in the present dataset is mainly due to biological variation rather than methodological variation. However, further clinical research is necessary to assess whether inter- and intraobserver variations are within the clinically acceptable ranges.

Reference ranges of the ductus venosus in the fetus have been needed for many years in Japan. In this study, we established reference ranges for the PI and a/S of ductus venosus waveform indices of fetuses in the Japanese population. Further clinical research is necessary to determine the validity of the clinical importance of the ductus venosus in at-risk fetuses.

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Fulminant type 1 diabetes mellitus acutely emerged during pregnancy

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Abstract

A pregnant woman at 32 weeks of gestation was emergently admitted to our hospital with symptoms of nausea, vomiting, and uterine contraction. Cardiotocogram demonstrated a loss of variability and late deceleration in fetal heart rate pattern. Emergency cesarean section was performed, and a male infant weighing 1750 g was born with Apgar scores of 1 at 1 min, and 3 at 5 min after delivery. After cesarean section, the patient developed an acetone breath odor, and blood examination demonstrated remarkable acidemia and an extremely high level of blood glucose. The patient was diagnosed with ketoacidosis with acute onset of fulminant type 1 diabetes mellitus. Intensive care was applied due to the severe diabetes mellitus conditions. The patient's general condition ameliorated during the postoperative period, although there was a possibility of neurological complications in the infant.

Key words: cesarean section, critical care obstetrics, diabetes mellitus.

Introduction

Fulminant type 1 diabetes mellitus, which is included in type 1B diabetes, accounts for approximately 20% of cases of acute-onset type 1 diabetes, and is recognized as a novel subtype of type 1 diabetes.[1,2] The major clinical characteristics of fulminant type 1 diabetes mellitus are markedly abrupt onset of disease, very short (<1 week) duration of diabetic symptoms, and severe acidosis at the time of diagnosis. A recent 5-year nationwide survey performed in Japan reported 18 cases of fulminant type 1 diabetes mellitus that emerged during pregnancy, and that the infants were rescued in only six cases (approximately one per year).[3] We report the clinical course of a pregnant woman with typical fulminant type 1 diabetes mellitus who had an extremely acute onset of symptoms and whose baby was rescued.

Case Report

A pregnant woman at 32 weeks of gestation was emergently admitted to our hospital with symptoms of nausea, vomiting, and uterine contraction. Slight symptoms, such as thirst and polyuria, began roughly 1 day before her emergency hospitalization. The patient received routine prenatal care and examinations from an obstetrician in another prefecture. She was visiting her parents' home near our hospital when her symptoms occurred suddenly. Serial routine examinations had not revealed any urinary sugar, and a blood sugar examination performed at 28 weeks of gestation showed a normal value. The patient had no family history of diabetes mellitus, nor any past history of the disease.

On admission, the patient was alert and no sign of coma was observed. Cardiotocogram demonstrated a

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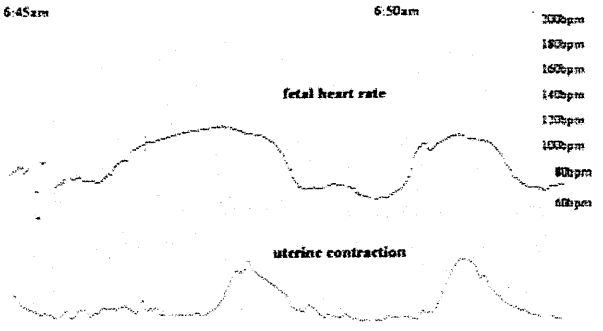

Figure 1 Cardiotocogram of the patient, examined just after emergency admission. Loss of variability and late deceleration were noted.

loss of variability and late deceleration in the fetal heart rate pattern (Fig. 1). Emergency cesarean section was performed, and a male infant weighing 1750 g was born with Apgar scores of 1 at 1 min after delivery, and 3 at 5 min. After the cesarean section, the patient developed an acetone breath odor, and blood examination demonstrated marked acidemia and an extremely high blood glucose level.

Results of laboratory examinations performed just after the surgery were as follows:

- Complete blood count: white blood cells 22 920/μL; red blood cells 461 × 10^4/μL; hemoglobin 14.0 g/dL; hematocrit 42.6%, platelet 28.3 × 10^4/μL.
- Biochemical examination: blood sugar 557 mg/dL; total protein 8.5 g/dL; albumin 4.0 g/dL; blood urea nitrogen 22 mg/dL; creatinine 0.77 mg/dL; aspartate aminotransferase 39 IU/L; alanine aminotransferase 46 IU/L; lactate dehydrogenase 269 IU/L; Na 129 mEq/L; K 5.8 mEq/L; Cl 101 mEq/L; serum amylase 173 IU/L.
- Arterial blood gas analyses: pH 7.058; pCO2 13.8 mmHg; pO2 131.3 mmHg; HCO_3^- 3.8 mmol/L; base excess −24.5 mmol/L; SaO2; 97.9%.
- Urinalysis: urinary sugar 4+; urinary acetone body 4+.

The patient was diagnosed with ketoacidosis and fulminant type 1 diabetes mellitus. The type of acidosis was determined to be metabolic because of low HCO_3^- and pCO2 values.

Results of laboratory examinations related to diabetes mellitus, performed during hospitalization, were as follows:

- Hemoglobin A1c 5.4%; urinary C peptide excretion 3.8 μg/day.
- Islet-related autoantibodies: antiglutamic acid decarboxylase (anti-GAD) antibodies negative; anti-islet cell (IC) antibodies negative; anti-islet antigen 2 (anti-IA2) antibodies negative.
- Serum exocrine pancreatic enzyme level: amylase 173 IU/L; lipase 269 IU/L.

Results of viral examinations performed at the third day after surgery were as follows:

- IgG to herpes simplex virus >128; IgG to cytomegalovirus >128; IgM to cytomegalovirus 0.46; IgG to Ebstein-Barr virus >160; IgM to Ebstein-Barr virus; <10; IgA to Ebstein-Barr virus <10; antibodies to poliovirus negative; antibodies to coxsachie virus negative; IgG to rubella virus 102; IgM to rubella virus 0.32; antibodies to measles ×8; antibodies to mumps ×16.

The genotype of human leukocyte antigens (HLA) of the patient was DRB1*0901/*1405 and DQB1*0303/*0503.

To treat the diabetes mellitus, rapidly acting recombinant human insulin was administered. First, 10 units of rapidly acting recombinant human insulin were injected intradermally several times, and thereafter 49.5 mL of physiological saline, including 50 units of rapidly acting recombinant human insulin, were continuously injected intravenously at a rate of 2 mL/h.

Blood glucose level decreased to 390 mg/dL on the night of surgery. Continuous insulin infusion was performed, and the glucose level decreased to within 250 mg/dL the next day. The sliding scale for using recombinant insulin was applied, and the blood glucose level improved close to the normal range. The general condition of the patient gradually ameliorated, and she was transferred to internal medicine on the 13th day after cesarean section. As for the neonatal condition, the results of umbilical arterial blood gas analyses were as follows: pH 6.660; pCO2 84.3 mmHg; pO2 13.5 mmHg; and BE −28.9 mmol/L; which indicated a severe acidemic and hypoxic condition in the infant. The infant was placed in the neonatal intensive care unit. The blood glucose level just after delivery was 294 mg/dL, and metabolic acidosis was adjusted by the infusion of sodium bicarbonate. Respiratory care was performed using high frequency oscillation with intubation. Surfactant was intratracheally administered for respiratory distress. The general condition of the infant gradually ameliorated, and artificial respiratory care ceased on the sixth day after birth. The infant was discharged on the 50th day after birth, with

the possibility of neurological complications due to hypoxic encephalopathy, as findings of periventricular leukomalasia was observed by ultrasonography and magnetic resonance image.

Discussion

Type 1 diabetes mellitus is characterized by insulin deficiency resulting from the destruction of pancreatic β-cells.[4,5] Type 1A diabetes mellitus involves direct damage to the pancreatic β-cells by islet-related autoantibodies,[6] and type 1B diabetes is considered to be idiopathic.[1] Of these, fulminant type 1 diabetes mellitus demonstrates the most severe clinical course.

The clinical characteristics of fulminant type 1 diabetes mellitus are as follows:[1,2] (i) markedly abrupt onset of disease; (ii) very short (<1 week) duration of diabetic symptoms (e.g. polyuria, thirst, and body weight loss); (iii) acidosis at the time of diagnosis; (iv) negative status of islet-related autoantibodies, such as islet cell antibodies, GAD ab, insulin autoantibodies, or IA-2ab; (v) virtually no C peptide secretion (<10 μg/day in the urine); and (vi) elevated serum pancreatic enzyme levels. The present patient fulfilled almost all of these criteria, therefore, she was diagnosed with typical fulminant type 1 diabetes mellitus.

The onset of the present case was markedly acute (i.e. no urinary sugar was detected on routinely performed prenatal examinations, and a blood sugar examination was normal at 28 weeks of gestation). Shimizu *et al.* compared the clinical characteristics between a patient group with pregnancy associated fulminant type 1 diabetes mellitus and non-pregnancy associated fulminant type 1 diabetes mellitus, and concluded that the clinical symptoms of pregnancy associated fulminant type 1 diabetes mellitus were more severe than non-pregnancy associated fulminant type 1 diabetes mellitus.[3,7] Recent reports have pointed to the possibility of viral infection, such as mumps and influenza, inducing fulminant type 1 diabetes mellitus.[8,9] Although several different viral examinations were performed, viral infection was not obvious in the present patient.

Serious systemic maternal diseases are related to onset of preterm uterine contraction.[10] In the present case, marked uterine contraction was observed on admission. Ketoacidosis during pregnancy decreases uteroplacental blood flow as the result of maternal hypovolemia and/or maternal acidemia itself.[11] Decreased uterine blood flow causes myometrial hypoxia, which provokes local inflammatory reaction and prostaglandin production. Such mechanisms are considered to have caused the manifestation of marked uterine contraction in the present case. Commonly used uterine relaxants, such as beta2 stimulant, will worsen diabetic hyperglycemia; therefore, careful management of uterine contraction must be applied in such cases.

In the present case, diabetic ketoacidosis was extremely marked at the onset of symptoms of fulminant type 1 diabetes mellitus. Diabetic ketoacidosis is a medical emergency during pregnancy because of maternal risks, such as manifestation of diabetic coma, as well as high rates of fetal mortality. Shimizu *et al.* reported that fetal demise occurred in 12 of 18 patients (67%) who developed fulminant type 1 diabetes mellitus during pregnancy, and cases demonstrating fetal demise showed more severe acidosis than patients with liveborn infants.[3] Montoro *et al.* reported that fetal loss was 35% in 20 patients with diabetic ketoacidosis during pregnancy,[12] although a lower percentage (9% of 11 cases) of fetal demise was reported by Cullen *et al.*[13] Montoro *et al.* demonstrated that new unrecognized onset diabetes accounted for 57% of fetal deaths compared with 21% in mothers with recognized disease.[12] Therefore, it is suggested that the abrupt onset of diabetes, as well as the severity of maternal diabetic ketoacidosis, might affect the high fetal mortality rate in pregnant women who develop fulminant type 1 diabetes mellitus.

The immunogenetic background of fulminant type 1 diabetes mellitus has been well analyzed (i.e. some susceptible human leukocyte antigens have been reported concerning the disease, especially pregnancy associated fulminant type 1 diabetes). Shimizu *et al.* demonstrated that the frequency of haplotype HLA-DRB1*0901-DQB1*0303 in pregnancy associated fulminant type 1 diabetes patients was 41.2%, which was significantly higher compared with non-pregnancy associated fulminant type 1 diabetes patients (17.5%), and controls (14.7%).[3] The HLA-class II genotype of our patient was DRB1*0901/*1405 and DQB1*0303/*0503, which strongly indicates that she had an immunogenetic predisposition to pregnancy associated fulminant type 1 diabetes.

As causes of the high fetal mortality rate in these pregnancies, various factors, such as maternal dehydration, which diminishes uteroplacental blood flow, and maternal acidosis leading to fetal acidosis, are considered relevant, although the precise mechanisms remain unclear.[11,14] Emergency cesarean section was performed in the present case to rescue the infant. Shimizu *et al.* reported that it may be possible for fetal lives to be

rescued if cesarean section, together with treatment for diabetic ketoacidosis is performed immediately after the development of diabetic ketoacidosis, as the duration of hyperglycemic symptoms tended to be shorter in cases of liveborn infants than in cases demonstrating fetal demise.[3]

As described above, a nationwide survey performed over 5 years in Japan indicated that there were 18 patients with fulminant type 1 diabetes mellitus emerging in the prenatal period, and the infants were rescued in only six of these cases (roughly one per year).[7] We encountered a pregnant woman with typical fulminant type 1 diabetes mellitus, whose infant was rescued by emergency cesarean section, although there was a possibility that the infant would have some neurological complications due to the acute onset of severe hypoxia in the fetus just before delivery. As slight symptoms, such as thirst and polyuria, commenced roughly 1 day before serious aggravation of the symptoms in the present case, it is possible that examinations for diabetes mellitus at the manifestation of slight symptoms could predict the onset of the fulminant type 1 diabetes mellitus. In this context, it is crucial that women with fulminant type 1 diabetes mellitus emerging during pregnancy be recorded and the clinical course of these patients be analyzed. Moreover, all physicians as well as obstetricians should recognize and treat fulminant type 1 diabetes mellitus as soon as possible if a pregnant women abruptly develops hyperglycemic symptoms.

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Fetal Atrioventricular Block and Postpartum Augmentative QT Prolongation in a Patient with Long-QT Syndrome With KCNQ1 Mutation

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2:1 AV Block in KCNQ1. **The case of a 32-year-old pregnant woman, who had had several syncopal episodes during swimming and running at 9 and 10 years of age and whose fetus had 2:1 AV block, is presented. The mother and baby had the same heterozygous single nucleotide substitution in KCNQ1 at T587M. After 27 weeks of gestation, the fetal 2:1 AV block disappeared, and 1:1 AV conduction resumed, with a fetal heart rate of 110–120 beats/min. The maternal electrocardiogram revealed a normal QTc interval (433 ms) without ST-T abnormalities at gestational week 23, but the QTc was 490 and 531 ms at 1 and 2 months postpartum, with biphasic T waves in leads V2 and V3. This case is the first report of fetal 2:1 AV block with KCNQ1 mutation (T587M) and unmasked maternal QT prolongation in the postpartum period.** *(J Cardiovasc Electrophysiol, Vol. 21, pp. 1170-1173, October 2010)*

atrioventricular block, KCNQ1, long-QT syndrome, neonate, pregnancy

Introduction

The congenital long-QT syndrome (LQTS) is a potentially lethal cardiac disease associated with ventricular tachyarrhythmias, especially torsade de pointes, due to abnormally prolonged repolarization.[1,2] Bradyarrhythmias during the fetal or perinatal stages may be associated with 2:1 atrioventricular (AV) block and carry a worse prognosis.[3,4] Previous reports of LQTS with 2:1 AV block have been related to homozygous mutations in HERG[5,6] or SCN5A.[7]

On the other hand, women with LQTS have a reduced risk for cardiac events during pregnancy, but an increased risk during the postpartum period, especially women with HERG and KCNQ1[8]; however, the change in the QT interval in the peripartum period is not yet well elucidated.

A case of maternal postpartum augmentative QT prolongation, whose fetus had 2:1 AV block associated with KCNQ1 mutation, is presented.

Case Report

A 32-year-old pregnant woman was referred to our clinic because her fetus had 2:1 AV block. Fetal echocardiography showed normal growth and cardiac structure, but the superior vena cava (SVC)/ascending aorta (AA) Doppler tracing showed 2:1 flow velocities, representing 2:1 AV block (Fig. 1A). The patient had had several syncopal episodes during swimming and running at 9 and 10 years of age, but she did not have a medical check-up. There was no family history of sudden death. Her chest X-ray, blood chemistry data, and cardiac echocardiography were normal. Autoantibody screening was negative for antinuclear antibodies anti-SS-A, anti-SS-B, anti-Sm, and anti-RNP. An electrocardiogram (ECG) revealed normal sinus rhythm at 63 beats/min, and the QTc interval was 433 ms without ST-T abnormalities at gestational week 23 (Fig. 2A). After gestational week 27, the fetal 2:1 AV block disappeared, and the fetal heart rate increased to 110–120 beats/min with 1:1 AV conduction. She had childbirth by a Caesarean operation week 38 of gestation (Apgar score: 7/9). At the age of 1 week, the neonate's QTc interval prolonged to 521 ms on ECG (Fig. 1B), but in the neonate's cardiac echocardiography there was no abnormality and the ejection fraction of the left ventricle was 67%. No arrhythmia including 2:1 AV block nor sinus bradycardia was seen in the infant period. The baby was given carteolol at a dose of 0.2 mg/kg/day from the age of 3 months. The mother's ECG showed QTc interval prolongation to 490 ms 1 month postpartum (Fig. 2B) and to 531 ms 2 months postpartum; in particular, biphasic T waves appeared in leads V2 and V3 (Fig. 2C). The mother was prescribed propranolol at a dose of 30 mg/day.

DNA Isolation and Mutation Analysis

Genomic DNA was isolated from leukocyte nuclei obtained from the mother and baby by conventional methods.[9] Direct PCR was performed for all exons$^{1-15}$ of KCNQ1 isoform 1.[10] The primers were constructed, and all PCR products were sequenced in both directions using the same primers as used for the first round PCR.[10] DNA sequencing was performed by an ABI-Prism 310 DNA sequencer (Perkin-Elmer/Applied Biosystems, Foster City, CA, USA) using BigDye terminator premix reagent. The mutations previously reported in HERG, SCN5A, KCNE1, and KCNE2 were also screened in the same way.

Results of Mutation Analysis

In the mother and neonate, the DNA sequencing identified the same heterozygous single nucleotide substitution in KCNQ1 at position 1760 (C to T), resulting in an amino acid substitution

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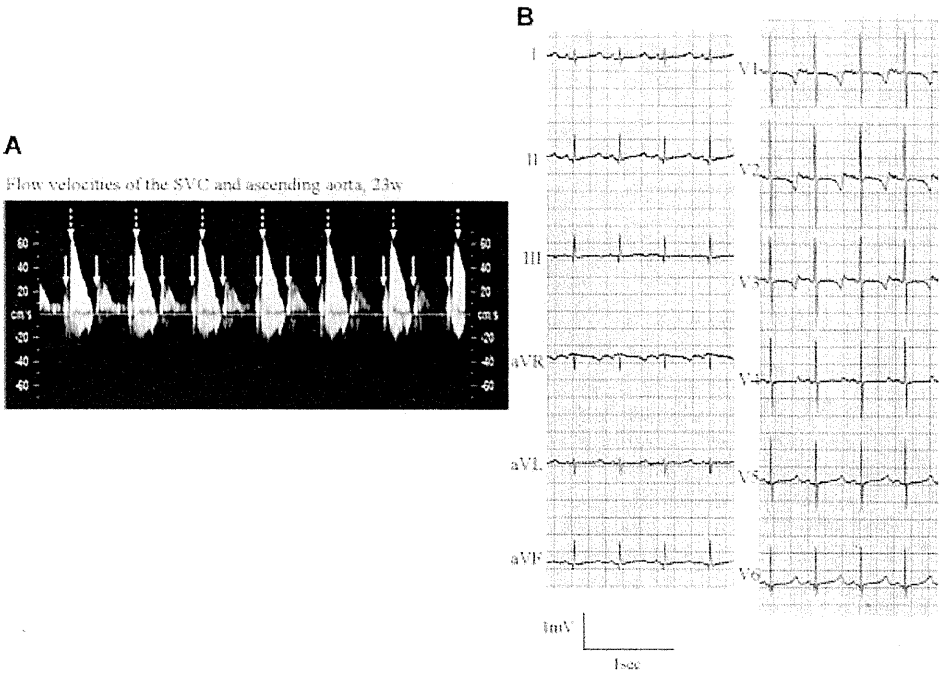

Figure 1. *(A) Flow velocities of the SVC and AA at gestational week 23, suggesting 2:1 AV conduction block. Solid arrows indicate reverse flow of the SVC, and dotted arrows indicate forward flow of the AA. (B) A 12-lead surface ECG of the newborn child at the age of 1 week. The corrected QT interval (QTc) at a sinus rate of 114/min is 521 ms.*

of methionine for threonine at codon 587 (T587M), which has been previously reported (Fig. 3A).[11] This heterozygous mutation was located in the C-terminal domain of KCNQ1 (Fig. 3B). The mutated alleles were also confirmed by restriction analysis with *PfuI*. The same nucleotide substitution was not observed in greater than 100 normal individuals.[11] Direct sequencing of other primer sets for KCNQ1, HERG, SCN5A, KCNE1, and KCNE2 revealed no mutations.

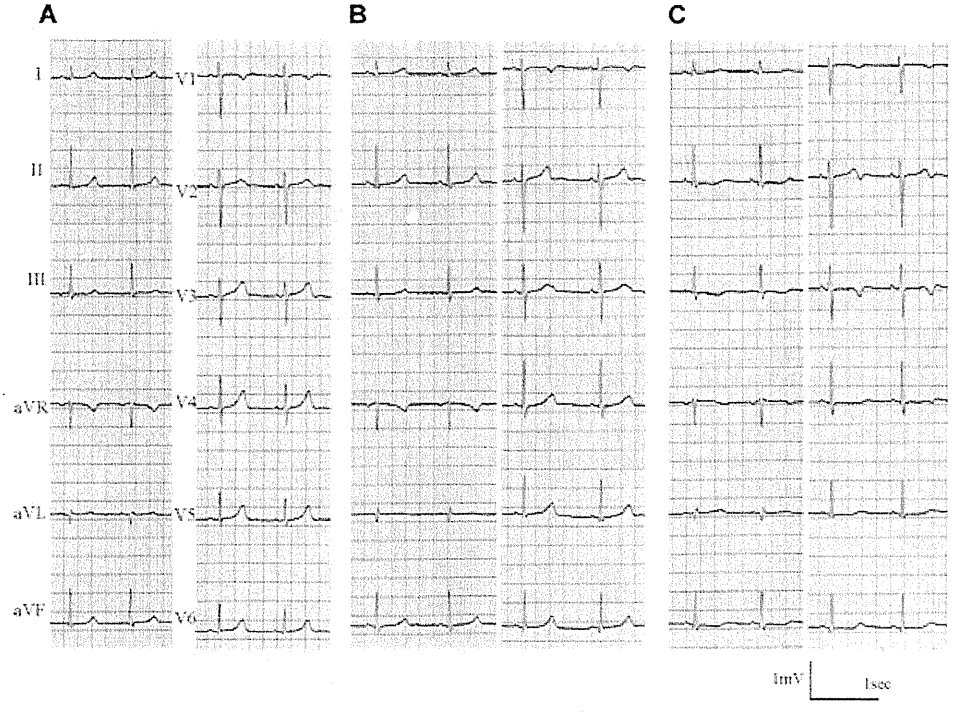

Figure 2. *A 12-lead surface ECG of the mother. The QTc interval is in the normal range (433 ms) without ST-T changes at gestational week 23 (A). The QTc interval is prolonged to 490 ms at 1 month postpartum (B) and to 531 ms at 2 months postpartum, with a biphasic T wave abnormality in leads V2 and V3 (C).*

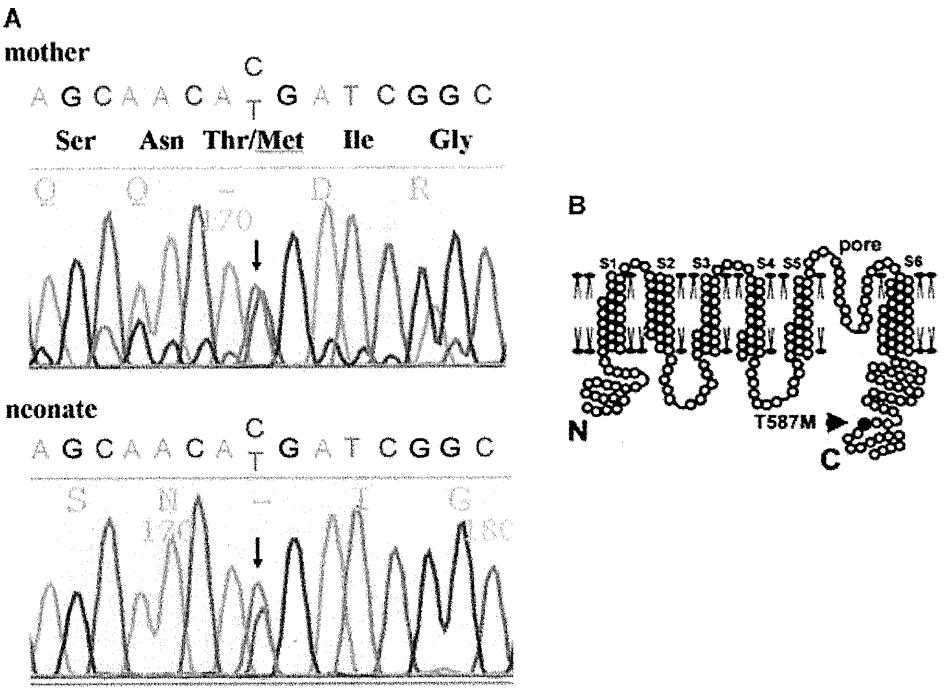

Figure 3. *(A) Electropherogram of the sequence around the heterozygous mutation in the KCNQ1 coding region from the mother (upper panel) and neonate (lower panel). The mother and neonate had the same mutation. An arrow denotes the heterozygous point mutation (C to T transition at position 1760 of the KCNQ1 cDNA sequence, underlined). Amino acid residues are listed under their corresponding nucleotide codons; note that this mutation results in the substitution of a methionine for a threonine at codon 587 (T587M). (B) Schematic location of the T587M in the KCNQ1. S1 to S6 refer to the putative transmembrane domains.*

Discussion

We demonstrated fetal 2:1 AV block with KCNQ1 mutation (T587M). So far, little is known about the genotype of patients with LQTS and 2:1 AV block. Previous reports of LQTS with 2:1 AV block have been related to homozygous mutation in HERG[5,6] or SCN5A,[7] although it was reported that persistent sinus bradycardia is associated with KCNQ1 mutations. To the best of our knowledge, this case is the first report of fetal 2:1 AV block with KCNQ1 mutation (T587M). Ventricular tachyarrhythmias during the fetal or perinatal stages may be associated with functional AV block and carry a worse prognosis.[12] In the presented case, fetal 2:1 AV block could be associated with marked QT prolongation. QT prolongation was evident at the age of 1 week.

A T587M is a mutation in the KCNQ1 C-terminal domain that causes a trafficking defect and a nonfunctional channel without a dominant-negative effect and a severe form that differs from other previously reported C-terminal mutations.[11] In the previous reports, patients with T587M began to suffer from repeated syncopal attacks and/or sudden death during exercise at 7–10 years.[11,12] Although this period seems to be high risk of cardiac event in patients with T587M, we think that persistent care is very important in the presented child, and we have prescribed β-blockade since the age of 3 months and should inform and educate parents about the child's daily life activity including limitation of certain exercise (e.g., swimming).

The ECG of the mother showed a normal QTc interval at gestational week 23. However, the QTc interval was prolonged with a biphasic T wave in the postpartum period. Whether the prolongation of the QT interval was masked during pregnancy or the QT interval deteriorated in the postpartum period is unknown because her ECG was not taken before pregnancy. In females, there are dynamic fluctuations in the QT interval and torsade de pointes risk during the menstrual cycle and pregnancy. The repolarization duration of the ventricle is relatively shorter in the luteal phase than in the follicular phase by $\approx$10 ms.[14] This suggests that sex hormones can be related to ventricular repolarization. Seth *et al.* recently reported that women with KCNQ1 and HERG mutations have a reduced risk for cardiac events during pregnancy, but an increased risk during the 9-month postpartum period, especially HERG mutation;[8] however, the change in the QT interval between pregnancy and postpartum and its relationship to outcome was not well investigated. Estrogen and progesterone levels are high during pregnancy and decrease well below normal levels when the mother breastfeeds her child. Seth *et al.* speculated that the hyperestrogenic state during pregnancy may be a factor associated with a reduced risk of cardiac events during this time period. However, it was previously reported that estrogen prolonged the QT interval,[15,16] which is contradictory to the results of the present case. Other factors that might be related to QT prolongation in the postpartum period in this case, such as lactation, adrenergic effects, and the response to these, depend on the genotype. Recently, it was reported that progesterone shortened the action potential duration of pig ventricular myocytes associated with enhancement of the slow delayed rectifier K^+ current (I_{Ks}).[17] Furthermore, in postmenopausal women, hormone replacement therapy with estrogen prolongs the QTc interval, whereas combinational hormone replacement therapy with estrogen and progestin consistently shortens the QTc interval.[18] These studies suggest that the ratio of

progesterone to estrogen may be crucial to the QT interval. Further studies are required, including examination of the relationship between the change in the QT interval and the peripartum period.

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Case Report

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A Case Study of a Pregnant Patient with a Congenital Heart Block Accompanied by Left Isomerism and Uncontrolled Type 2 Diabetes Who Was Treated Successfully with Ritodrine

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Key Words

Congenital heart block · Left isomerism · Ritodrine hydrochloride · Type 2 diabetes mellitus

Abstract

We present a case study of a patient with a congenital heart block associated with a left isomerism that was diagnosed during the 26th week of gestation. The mother had type 2 diabetes mellitus that was difficult to control during the early stages of the pregnancy. A fetal echocardiogram revealed an atrioventricular dissociation, with an atrial rate of 120 bpm and a ventricular rate of 55 bpm. Subsequent examinations also revealed a left isomerism in the fetus. To increase the fetal heart rate, a continuous intravenous infusion of ritodrine was administered. The fetal ventricular rate rapidly increased to 65 bpm. The pregnancy successfully continued until term and a female infant weighing 3,182 g was born via a cesarean section. A subsequent surgery was performed to provide the infant with a permanent cardiac pacemaker, and notably, the child is now 4 months of age and her growth has been within the normal range.

Case Report

A pregnant woman in the 26th week of gestation was referred to the Obstetric Outpatient Clinic of Niigata University Medical and Dental Hospital for close examination of fetal bradycardia and a suspicious left isomerism. She had been diagnosed with type 2 diabetes mellitus, which had manifested when she was 6 years old. The diabetes was poorly controlled and a concerned internal medicine doctor had initially advised against the pregnancy. She had, however, already conceived and desired to continue the pregnancy. The titer of glycosylated hemoglobin (HbA1c) during the early stages of pregnancy was determined to be 10.2%. She was then thoroughly informed that the risk of fetal anomalies significantly increased under such conditions, and ultimately she decided to continue with pregnancy. She underwent prenatal management and care at the hospital near her residence.

Ultrasonographic examinations performed during the first visit indicated that the fetal heart rate was 60 bpm, the atrial rate was 120 bpm and the ventricular rate was 60 bpm. Thus, a two-to-one atrioventricular block (AV block) was diagnosed. We also noted several cardiac abnormalities, a ventricle septal defect, a double outlet of the pulmonary artery and the aorta from the right ventricle, stenosis of the pulmonary valve, and a defect in the inferior vena cava. In addition, the cardiothoracic area ratio (CTAR) was about 30%. The fetus was diagnosed with a second degree AV block further characterized by a left isomerism.

She was hospitalized during the 28th week of gestation, and serial ultrasonographic examinations were performed, which

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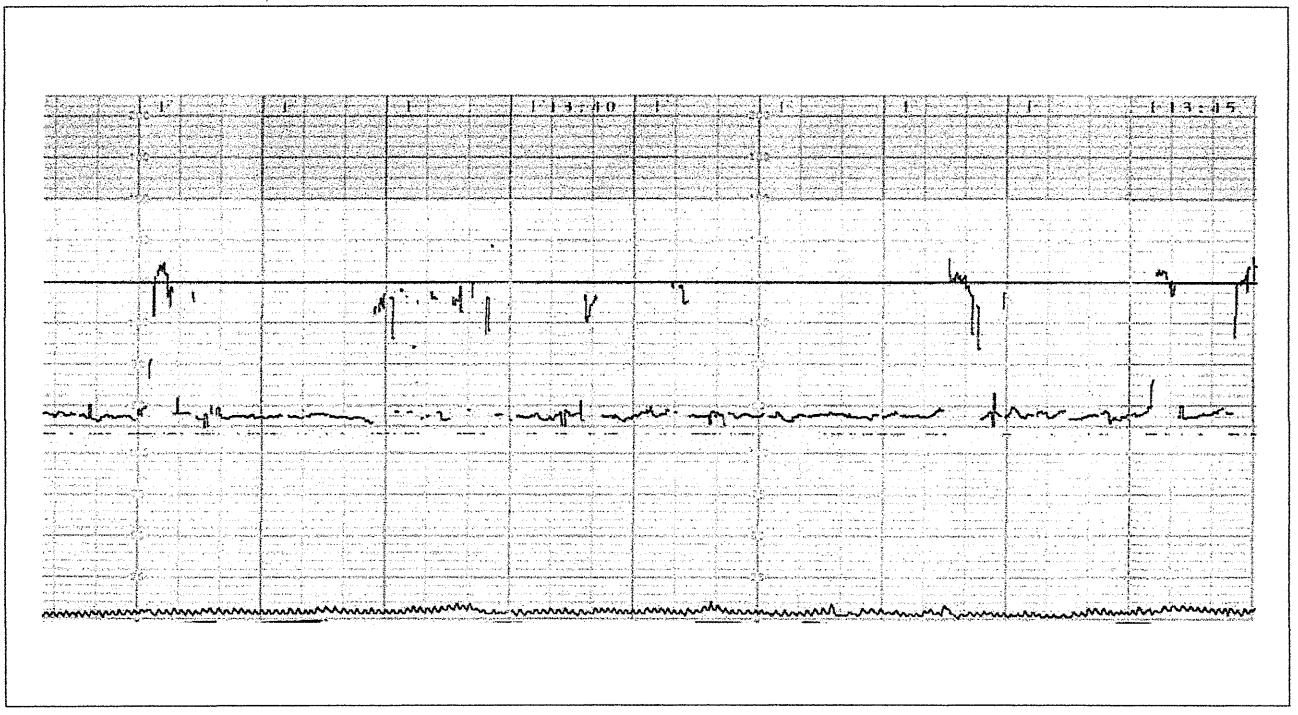

Fig. 1. Nonstress test performed during the 33rd week of gestation. Fetal heart rate was 55 bpm.

showed a stable fetal heart rate of about 60–70 bpm and revealed no cardiac enlargement and no fetal edema. Therefore, she was managed at the outpatient clinic of our hospital. However, during the 33rd week of gestation, the fetal heart rate decreased to 55 bpm (fig. 1), and the CTAR increased to 44%. She was subsequently readmitted to our hospital. Fetuses with a ventricular rate <55 bpm have been found to have a poorer prognosis than those with >60 bpm [1]. Since fetal heart rate had gradually decreased, we started to use the beta mimetic agent (ritodrine hydrochloride) to prevent hydrops fetalis so that it might not be less than 55 bpm. The fetal heart rate increased to 65 bpm upon infusion of ritodrine hydrochloride (fig. 2), and the CTAR was observed to be 30%, which indicated no cardiac failure in the fetus. Administration of ritodrine hydrochloride intravenously was continued until the 37th week of gestation. There were no maternal complications by ritodrine. The blood sugar level was kept from 100 to 150 mg/dl under insulin treatment.

It would be very difficult to evaluate CTG monitor because of fetal advanced bradycardia when the labor was onset. We decided to perform a cesarean section, as a result of consulting with her and her husband about a delivery mode. She underwent elective cesarean section during the 37th week of gestation, and a female infant weighing 3,182 g was delivered with an Apgar score of 6 and 7 at 1 and 5 min postdelivery, respectively. The postoperative course of the patient was fairly uneventful such that she was discharged from our hospital on the 8th day after the operation. Her diabetes mellitus was well controlled using recombinant human insulin administered throughout the second and third trimester. After delivery, the infant was confirmed to have cardiac anomalies such as double outlet of the pulmonary artery and the aorta from the right ventricle, atresia of the pulmonary valve, a defect in the inferior vena cava, and patent ductus arteriosus. In addition, a left isomerism was diagnosed. On the other hand, there were no side effects by ritodrine to the neonate as written in the pharmaceutical references. A permanent pace maker was introduced into the fetus for normalizing the infant's bradycardia, and the heart rate was maintained at 120 bpm after the surgical procedure. The infant's weight gain was observed to be normal.

Disussion

It is generally believed that poorly controlled diabetes during the early stages of pregnancy results in an increased risk of congenital malformations. Fuhrmann et al. [2] reported that there was only one malformation observed in 128 infants (0.8%) of diabetic women who, while planning pregnancy, underwent intensive treatment before conception. They also reported that 22 of 292 diabetic women (7.5%), for whom strict metabolic control was initiated as late as after the 8th week of gestation, de-

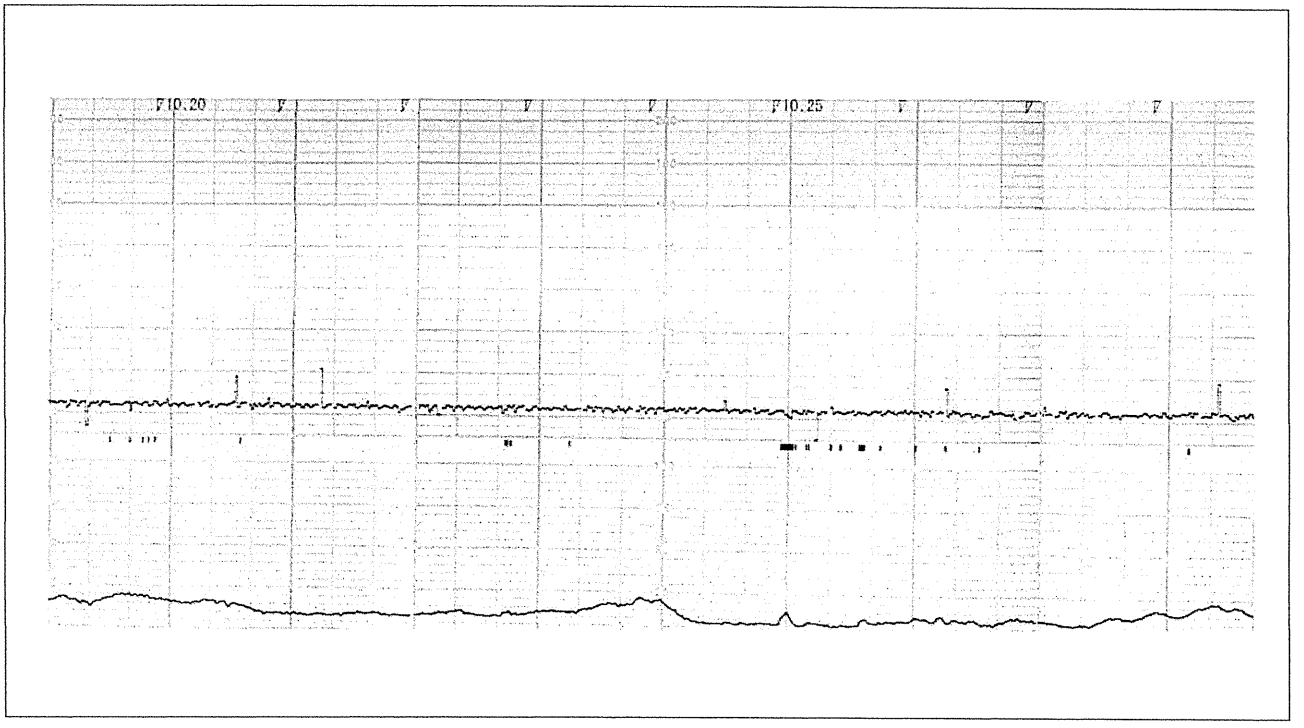

Fig. 2. Nonstress test performed after the administration of ritodrine hydrochloride. Fetal heart rate improved to 65 bpm.

livered infants with congenital malformations, an observation that is suggestive of a close relationship between the generation of congenital malformations and the poor management of diabetic mellitus. In the index case, the titer of HbA1c during the early stage of the pregnancy was 10.2% and as such, poorly controlled diabetes was possibly the cause of the fetal heart malformations. Isomerism sequence, bilateral right-sidedness or left-sidedness, is a rare defect with an estimated birth prevalence of approximately 1 in 24,000 individuals. A specific association between left-isomerism sequence and maternal type 1 diabetes has been suggested [3]. However, the association with type 2 diabetes has not been well established.

The current case demonstrated a complete AV block accompanied by left isomerism and multiple cardiac anomalies. It has been reported that the prognosis of AV block accompanied by structural heart disease is very poor, particularly with atrial isomerism, as a result of the disruption of the early left-right axis determination [4]. In addition, the prognosis of a fetus with congenital heart block is not always favorable. A higher mortality rate has been documented in cases that demonstrated a structural anomaly of the heart, hydrops fetalis, or a ventricular rate less than 55 bpm [1, 4]. We previously reported a case of a congenital heart block associated with the maternal anti-SSA antibody, in which maternal administration of ritodrine hydrochloride was observed to increase the fetal heart rate from 54 bpm to 65 bpm, and the pregnancy successfully continued to term [5]. Maternal administration of beta mimetic agent has been proposed to increase fetal ventricular rate [6]. We started maternal ritodrine infusion because it has been used for the treatment of threatened premature delivery most commonly in our country and we are familiar with the maternal and fetal side effects of ritodrine such as pulmonary edema, arrhythmia, pancytopenia, liver dysfunction, rhabdomyolysis and neonatal hypoglycemia.

Therefore, we administered ritodrine hydrochloride to increase the fetal heart rate in this case. The fetal heart rate was observed to be 55 bpm during the 33rd week of gestation. We consulted with pediatric cardiologists and they said termination of the pregnancy would be desirable when the fetal bradycardia advanced and hydrops fetalis developed, and the fetus at the 33rd week of gestation was too small to embed a pacemaker. They asked us to try ritodrine use for prevention of hydrops fetalis. Ma-

ternal intravenous ritodrine hydrochloride infusion was performed, after which the heart rate rapidly increased to 65 bpm. As we tried to detect side effects of ritodrine with pulse oximeter and examining blood test, no complications were detected. The administration of ritodrine hydrochloride was continued until term because maternal diabetes was controlled well under internal medicine management, and a mature female infant was delivered without neonatal hypoglycemia.

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GAMETE BIOLOGY

Increased apoptosis of germ cells in patients with AZFc deletions

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Abstract
Purpose AZFc deletions are associated with variable testicular histology ranging from the Sertoli cell only to spermatogenic arrest and hypospermatogenesis. Such variable phenotypes may be explained by progressive germ cell regression over time. Increased apoptosis is likely responsible for progressive regression of spermatogenic potential. This study evaluated germ cell apoptosis as a cause of the progressive decrease in the number of germ cells in patients with AZFc deletions.
Methods This study evaluated germ cell apoptosis in patients with AZFc deletions. A total of 151 patients who were diagnosed with either severe oligozoospermia or non-obstructive azoospermia were screened for Y chromosome microdeletions. Germ cell apoptosis was examined using terminal deoxy-nucleotidyl transferase-mediated digoxigenin-dUTP nick-end labeling (TUNEL) on formalin-fixed 5-μm sections of testicular specimens.
Results Seven out of 117 (6.0%) patients with azoospermia and 4 of 34 (11.8%) patients with severe oligozoospermia had Y chromosome microdeletions. The percentage of apoptotic germ cells in the testes of patients with AZFc deletions were significantly increased compared to those of patients without AZFc deletions.
Conclusions These results suggest that increased apoptosis of germ cells is responsible for the progressive decline of spermatogenic potential in patients with AZFc deletions.

Keywords Apoptosis · AZF genes · Germ cells · Inhibin B · Microdeletions

Capsule Males carrying AZFc deletions exhibit diminished sperm cell numbers due to an enhanced incidence of apoptosis.

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Introduction

AZFc deletions are the most frequent genetic cause of male infertility, observed with a prevalence of 10–15% in patients with severe oligozoospermia and azoospermia [1]. The DAZ gene family is thought to be the major candidate responsible for the AZFc phenotype. The DAZ gene encodes a protein with an RNA-binding domain that is expressed exclusively in germ cells [2]. The natural RNA substrates of DAZ proteins remain undefined, and the biological function of DAZ has not yet been elucidated.

AZFc deletions are associated with variable testicular histology, ranging from the Sertoli cell only to spermatogenic arrest and hypospermatogenesis. A possible explanation for such variable phenotypes is the progressive germ cell regression over time, which has been reported in patients with AZFc deletions [3–8].

The control of germ cell apoptosis plays an important role during normal spermatogenesis [9–12]. Increased apoptosis can induce a progressive decrease in the number of germ cells. No studies have thus far assessed the apoptosis of germ cells in patients with AZFc deletions. Therefore, the current study evaluated germ cell apoptosis as one of the causes of the progressive decrease in the number of germ cells in patients with AZFc deletions.

Materials and methods

Patients

A total of 151 patients who were diagnosed with severe oligozoospermia (sperm concentration of less than 1×10^6 per ml) or non-obstructive azoospermia were screened for Y chromosome microdeletions. Among these, 117 were azoospermics and 34 were oligzoospermics. Patients with iatrogenic azoospermia, varicocele or cryptochidism were excluded from this study. As controls, testicular samples were obtained from five patients with obstructive azoospermia who had normal spermatogenesis.

Specimens of bilateral testicular tissue were obtained by open biopsy. The biopsies were classified according to McLachlan *et al.* [13] as follows: hypospermatogenesis, all stages of spermatogenesis are present but reduced to a varying degree; germ cell arrest, the total arrest at a particular stage; Sertoli cell-only, no tubules containing germ cells. This study was approved by the hospital's Institutional Review Board and informed consent was obtained from all patients.

Y chromosome microdeletion assay

Genomic DNA was isolated from peripheral blood lymphocytes using standard procedures. Y chromosome microdeletions were evaluated using polymerase chain reaction of Y chromosome-specific STS markers. The STS markers used were as follows: AZFa: sY83, sY95, sY105; AZFb: sY118, sY126, sY136; AZFc: sY152, sY254, sY255, sY283.

In situ end labeling of testicular tissue sections

In order to detect apoptosis, terminal deoxy-nucleotidyl transferase-mediated digoxigenin-dUTP nick-end labeling (TUNEL) was performed on formalin-fixed 5-μm tissue sections of specimens using an In Situ Apoptosis Detection Kit (Takara Bio Inc., Shiga, Japan). In brief, each section was deparaffinized and rehydrated. After incubation with 20 μg/ml Proteinase K (Boeheringer Mannheim, Mannheim, Germany), endogenous peroxidase were blocked with 2% H_2O_2 in methanol for 30 min. TdT enzyme was dropped on the sections and incubated at 37°C for 60 min. Then antifluorescein isothiocyanate horseradish peroxidase conjugate was placed on the sections and incubated at 37°C for 30 min. Slides were washed three times in PBS, developed with 0.05% diaminobenzidine (DAB), and stained for 10–15 min at room temperature. The specimens were then washed three times in distilled water, dehydrated and mounted. For quantitative evaluation, the percentage of labeled cells per total 200 cells of germ cells was evaluated for each patient.

Hormone assays

Semen samples were centrifuged (3000× g; 5 min) and the seminal plasma was stored at –20°C within one hr after ejaculation. Inhibin B was measured by two-site enzyme-linked immunoassay (Serotec Ltd., Oxford, UK).

Statistical analysis

The Mann-Whitney U test was used for statistical analyses using the StatView 5.0 statistical analysis program (Abacus Concepts, Berkeley, CA, USA). Statistically significant differences were confirmed for p values less than 0.05.

Results

Seven out of 117 (6.0%) patients with azoospermia and 4 of 34 (11.8%) patients with severe oligozoospermia had Y chromosome microdeletions (Table 1). AZFa, AZFb and AZFc were deleted in two azoospermic patients. AZFb and AZFc were deleted in one azoospermic patient. AZFc was deleted in four azoospermic patients and in four severe oligozoospermic patients. All patients with AZFa+b+c and AZFb+c deletions had a complete absence of spermatozoa upon testicular biopsy. Of the 8 patients with AZFc deletions, 6 had spermatozoa within the testis or ejaculate.

Serum and seminal plasma Inhibin B were undetectable in patients who lacked testicular spermatozoa. The seminal plasma Inhibin B level was greater than 15 pg/ml in all patients who had spermatozoa in testes or ejaculate (Table 2). Sequential seminological data was available in two patients with AZFc deletions. Patient 4 showed a

Table 1 Summary of DNA analysis of the twelve patients with Yq microdeletions

Markers	Patients										
	1	2	3	4	5	6	7	8	9	10	11
sY83	+	+	+	+	+	+	+	+	+	+	+
sY95	–	+	+	+	+	+	+	+	+	+	+
sY105	–	+	+	+	+	+	+	+	+	+	+
sY118	–	–	+	+	+	+	+	+	+	+	+
sY126	–	–	–	+	+	+	+	+	+	+	+
sY136	–	–	–	+	+	+	+	+	+	+	+
sY152	–	–	–	–	–	–	–	–	–	–	–
sY254	–	–	–	–	–	–	–	–	–	–	–
sY255	–	–	–	–	–	–	–	–	–	–	–
sY283	–	–	–	–	–	–	–	–	–	–	–
sY166	+	+	+	+	+	+	+	+	+	+	+