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Relationship between single nucleotide polymorphisms in *CYP1A1* and *CYP1B1* genes and the bone mineral density and serum lipid profiles in postmenopausal Japanese women taking hormone therapy

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Abstract

Objective: The genetic variations of the genes encoding cytochrome P-450 enzymes are considered to play an important role in the metabolism of estradiol. The objective of this study was to evaluate the relationships among single nucleotide polymorphisms (SNPs) of cytochrome P-450 genes, lumbar bone mineral density (BMD), and serum lipids and to determine the effects of hormone therapy (HT).

Design: The participants were 124 Japanese women who had been diagnosed with osteopenia or osteoporosis and were taking HT for 12 months. Seven single nucleotide polymorphisms in the *CYP1A1* and *CYP1B1* genes were characterized. Lumbar BMD and the levels of serum lipids were measured before and after HT.

Results: A single nucleotide polymorphism in exon 3 of *CYP1B1* was found to be significantly associated with the effect of HT on BMD and low-density lipoprotein cholesterol both in univariate and multivariate analyses. In the women with the GG genotype of L432V, the responses to HT of BMD and low-density lipoprotein cholesterol markedly decreased. The serum follicle-stimulating hormone level after HT was significantly higher in the women with the GG genotype of L432V.

Conclusions: These results suggest that the L432V polymorphism in the *CYP1B1* gene could therefore be used to predict the effect of HT on lumbar BMD and low-density lipoprotein cholesterol in Japanese women.

Key Words: Single nucleotide polymorphism – *CYP1A1* – *CYP1B1* – Hormone therapy – Bone mineral density – Low-density lipoprotein cholesterol – Follicle-stimulating hormone.

Estrogen plays a significant role in bone and lipid metabolism, and its deficiency after menopause is the main reason for accelerated bone loss and deterioration of the serum lipid profiles, which are preventable by estrogen administration. A number of observational studies have suggested that hormone therapy (HT) reduces the risk of fractures and coronary events in postmenopausal women.[1-4] However, recently published results from randomized clinical trials of HT indicate that this therapy does not slow the progression of coronary atherosclerosis, whereas the reduction in the hip and clinical vertebral fracture rate is significant.[5,6] Our understanding is limited regarding why not all women benefit from such therapy. However, it is still possible that a genetically determined subgroup of the population could benefit from this therapy.

Postmenopausal HT is generally an effective treatment modality to prevent bone loss while also improving the serum lipid profiles; however, individual variations exist.[7-9] Some postmenopausal women respond strongly to HT, whereas approximately 8% who are compliant with this therapy are nonetheless nonresponders.[2] This raises the possibility that some genetic determinants as well as gene-environment interactions might modulate the responses to HT in individual participants.

Individual genetic variability of estradiol metabolism has been described as a significant contributor to the disease susceptibility with variations depending on ethnic background. Among others, the genetic variations of the genes encoding cytochrome P-450 (CYP) enzymes are considered to play an important role in this regard.[10] CYP enzymes play an important role in the production, bioavailability, and degradation of estradiol. A series of polymorphisms and mutations of the CYP enzyme complex have been identified. *CYP1A1* and *CYP1B1* catalyze the hydroxylation of estradiol and several single nucleotide polymorphic sites of those genes have been described.[11,12] Polymorphisms, especially single nucleotide polymorphisms (SNPs) exist in the exon with amino acid changes, thus leading to functionally relevant biochemical consequences that are therefore capable of influencing the responses to HT.

In this study, we attempted to clarify whether SNPs in the exons of the *CYP1A1* and *CYP1B1* genes affected the change in bone mineral density (BMD) and serum lipid profiles in postmenopausal Japanese women during HT.

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METHODS

Design

The participants were 124 Japanese women, ranging in age from 40 to 64 years (49.8 ± 1.0 y, mean ± SEM) who had been diagnosed with osteopenia or osteoporosis and were willing to take HT for 12 months. The diagnoses of osteopenia and osteoporosis were based on the criteria recommended by the Japanese Society of Bone and Mineral Research: a lumbar BMD (L2-4) of less than 80% and less than 70% in younger adults (20-44 y), respectively. In all cases, more than 6 months had elapsed since the last menstrual period, the serum estradiol level was lower than 20 pg/mL, and the serum follicle-stimulating hormone (FSH) level was more than 50 mIU/mL. The exclusion criteria were a history of metabolic disease (including hyperparathyroidism, previously diagnosed osteoporosis, or nontraumatic vertebral fracture on baseline radiograph), chronic disease (uncontrolled hypo- or hyperthyroidism, liver disease, or unstable cardiac disease), cancer or thromboembolic disease, a history of treatment with glucocorticoids for more than 6 months, current HT use or HT use within the past 3 months, or a metabolic or other endocrine disease that could influence lipid metabolism. None of the women smoked or drank alcohol to excess, and none engaged in regular strenuous exercise. Furthermore, none had a history of illness or medical therapy, apart from HT, that might affect bone turnover or lipid metabolism. The women were not genetically related. HT was administered either in a sequential regimen (50 women) consisting of 0.625 mg conjugated equine estrogens for 24 days (days 1-24) and 5 mg medroxyprogesterone acetate for 10 days (days 15-24) or a continuous regimen (74 women) consisting of 0.625 mg conjugated equine estrogens and 2.5 mg medroxyprogesterone acetate for 28 days, according to the woman's preference.

Measures

Bone densitometry

BMD, expressed as the mass per unit area (g/cm^2), was measured in the anteroposterior plane of the lumber spine (L2-4), using dual-energy x-ray absorptiometry with a QDR-2000 analyzer (Hologic Inc., Waltham, MA); absorptiometries were examined by the same observer. The average coefficients of variation of the phantom measurements of bone mineral content, bone area, and BMD during the study period were 1.1%, 0.7%, and 0.6%, respectively. In addition, in the control women, the coefficient of variation of the in vivo precision of BMD between two measurements (mean interval: 2.6 ± 1.2 y) was 0.9%. There was no scanner drift observed during the study period. BMD change (ΔBMD) was expressed as the percentage of BMD change compared with the pretreatment baseline.

Analysis of lipids

After an overnight fast (a minimum 12-h fast), blood was collected from each woman to estimate the lipids and lipoproteins. We measured the total cholesterol (Determiner L-TCN; Kyowa Medex, Tokyo, Japan) and triglyceride (L-type Wako TG-H; Wako Pure Chemical, Osaka, Japan) concentrations by enzymatic methods, and the high-density lipoprotein cholesterol concentration by a homogeneous method (Determiner L HDL-C, Kyowa Medex) using a Hitachi 7450 automated analyzer. Low-density lipoprotein cholesterol was calculated using Friedewald's equation.

Hormones and assays

The serum hormone levels were evaluated after 12 months of HT. Blood samples were drawn in the morning after an overnight fast. The serum was separated immediately and frozen at −80°C for future analysis. The hormone levels were measured using an electrochemiluminescent immunoassay for estradiol and a chemiluminescent immunoassay for luteinizing hormone (LH) and FSH. The hormone fractions were measured in three different batches, and a laboratory batch was also treated to determine the random effect in all hormone analyses. The sensitivity, expressed as the minimal detectable dose, was 11.0 pg/mL, 0.11 mIU/mL, and 0.06 mIU/mL for estradiol, LH, and FSH, respectively. The intra- and interassay coefficients of variation were 1.63% and

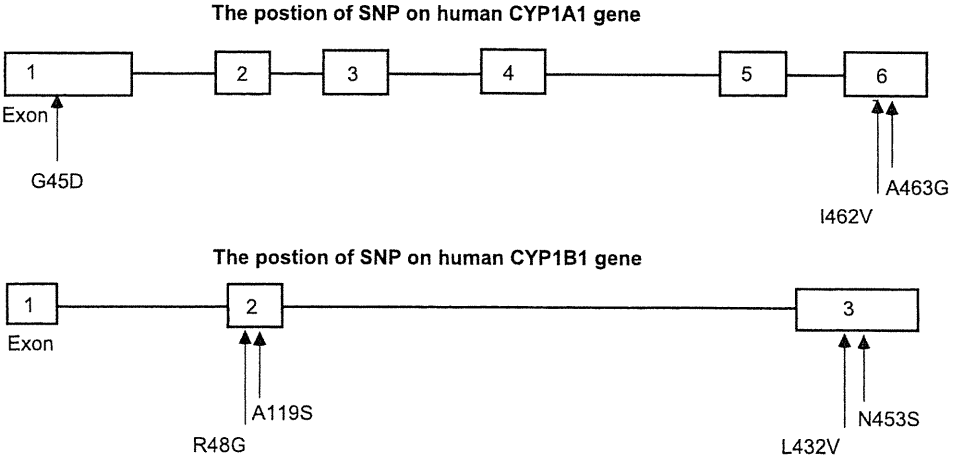

FIG. 1. The position of each single nucleotide polymorphism (SNP) in the *CYP1A1* and *CYP1B1* genes.

TABLE 1. *Genotype and allele frequencies of seven SNPs of CYP gene in Japanese participants*

Gene	Polymorphism	Genotype	Homo (major)	Hetero	Homo (minor)	Allele frequency (major allele) In this study	Sasaki et al	In dbSNP	JSNP ID	dbSNP
CYP1A1	G45D	GA	124 (100%)	0 (0%)	0 (0%)	1.00	—	—	ssj0003953	rs4646422
	I462V	AG	78 (62.9%)	42 (33.9%)	4 (3.2%)	0.80	—	0.902	ssj0007951	rs1048943
	A463G	CG	124 (100%)	0 (0%)	0 (0%)	1.00	—	—	IMS-JST026484	rs2278970
CYP1B1	R48G	CG	90 (72.6%)	16 (12.9%)	18 (1.5%)	0.79	0.68	0.653	ssj0007955	rs10012
	A119S	GT	124 (100%)	0 (0%)	0 (0%)	1.00	0.85	0.648	ssj0007956	rs1056827
	L432V	CG	78 (62.9%)	36 (29.0%)	10 (8.1%)	0.77	0.82	0.592	IMS-JST085313	rs1056836
	N453S	AG	124 (100%)	0 (0%)	0 (0%)	1.00	1.00	0.889	—	rs1800440

SNPs, single nucleotide polymorphisms; CYP, cytochrome P-450; Homo, homozygous; Hetero, heterozygous; JSNP, Japanese Single Nucleotide Polymorphism (database); dbSNP, Single Nucleotide Polymorphism database.

2.24% for estradiol, 3.37% and 3.62% for LH, and 3.50% and 5.28% for FSH.

DNA isolation and genotyping

The peripheral blood samples were collected after informed consent was obtained from each woman. Genomic DNA was extracted from the peripheral blood leukocytes using a DNA purification kit (QIAamp DNA Blood Mini kit; Qiagen, Valencia, CA) according to the manufacturer's instructions. All polymerase chain reactions were performed on a Perkin Elmer GeneAmp 9700 system, and the presence of amplicons was checked on agarose gel. A single nucleotide primer extension assay was performed to analyze SNPs using a SNaPshot Kit (Applied Biosystems, Foster City, CA). The extended primers were analyzed on an ABI 3100 device (Applied Biosystems). The primer sequences for the polymerase chain reactions and primer extension reactions are available in the Japanese Single Nucleotide Polymorphism database. Initial denaturation was performed at 95°C for 2 minutes, followed by 35 cycles each consisting of denaturation at 95°C for 30 seconds, annealing at 60°C, and extension at 72°C for 1 minute, followed by final extension at 72°C for 8 minutes. This study was approved by the Niigata University Human Investigation Committee.

Statistical analysis

Differences in the baseline characteristics, the absolute BMD value, and the serum lipid concentrations among genotypes were tested using an analysis of covariance with age and BMI as covariates. The values of triglycerides were not normally distributed and needed to be log-transformed for the statistical comparisons but, for clarity for presentation, the nontransformed values are presented in the text and tables. To evaluate the relationships between CYP polymorphisms and the change in BMD or serum lipid concentrations during HT, we used repeated-measures analysis of variance. A multiple linear regression model was used to evaluate the simultaneous contributions of different variables. Only those variables that had values of $P < 0.05$ in the univariate analysis were included in the multivariate analyses. All data are expressed as the mean ± SEM. Differences of $P < 0.05$ were considered to indicate statistical significance. All data management and statistical computations were performed with the StatView 4.0 (Abacus Concepts, Berkeley, CA) or the SPSS 10.0 software program (SPSS Inc., Chicago, IL).

RESULTS

In this study, we characterized seven SNPs, three SNPs in the *CYP1A1* gene and four SNPs in the *CYP1B1* gene, from a

TABLE 2. *Baseline characteristics according to the CYP genotypes*

	Genotype of I462V (*CYP1A1*)				Genotype of R48G (*CYP1B1*)				Genotype of L432V (*CYP1B1*)			
Variables	AA (n = 78)	AG (n = 42)	GG (n = 4)	*P*	CC (n = 90)	CG (n = 16)	GG (n = 18)	*P*	CC (n = 78)	CG (n = 36)	GG (n = 10)	*P*
Age, y	50.1 ± 0.8	49.2 ± 0.8	51.3 ± 1.8	0.73	49.6 ± 0.7	51.5 ± 1.4	49.4 ± 1.2	0.53	50.6 ± 0.8	49.0 ± 0.9	47.8 ± 1.4	0.74
Age at menopause, y	47.4 ± 0.6	47.7 ± 0.6	49.0 ± 1.9	0.89	47.6 ± 0.5	46.3 ± 1.6	48.3 ± 0.5	0.46	47.8 ± 0.6	47.2 ± 0.6	46.6 ± 1.9	0.39
Height, cm	154.9 ± 0.6	151.6 ± 2.4	157.8 ± 7.6	0.19	154.9 ± 0.6	154.9 ± 1.5	153.3 ± 0.9	0.51	153.6 ± 0.9	156.1 ± 1.2	158.5 ± 1.1	0.66
Weight, kg	52.5 ± 0.7	51.7 ± 1.0	51.0 ± 6.0	0.76	51.9 ± 0.7	53.4 ± 1.6	52.5 ± 1.6	0.64	52.0 ± 0.7	52.2 ± 1.2	53.2 ± 2.5	0.40
BMI, kg/m^2	21.9 ± 0.26	25.3 ± 3.5	20.3 ± 0.9	0.38	21.6 ± 0.2	22.3 ± 0.6	22.4 ± 0.6	0.34	23.7 ± 1.9	21.7 ± 0.4	22.1 ± 0.7	0.74
L2-4 BMD, g/cm^3	0.76 ± 0.02	0.76 ± 0.02	0.79 ± 0.08	0.96	0.76 ± 0.02	0.79 ± 0.05	0.75 ± 0.05	0.78	0.77 ± 0.02	0.76 ± 0.02	0.78 ± 0.07	0.23
TC, mg/dL	224.4 ± 4.2	227.2 ± 6.5	231.0 ± 13.5	0.89	223.8 ± 3.9	224.9 ± 8.1	234.5 ± 11.8	0.54	226.2 ± 4.3	228.6 ± 6.8	216.1 ± 10.9	0.65
LDL-C, mg/dL	132.6 ± 4.7	137.3 ± 5.9	132.5 ± 7.2	0.82	130.8 ± 3.8	140.1 ± 0.8	147.3 ± 13.0	0.24	136.1 ± 4.8	135.2 ± 6.2	123.9 ± 10.6	0.60
HDL-C, mg/dL	67.6 ± 1.9	67.0 ± 2.9	77.0 ± 6.8	0.51	67.5 ± 1.8	67.0 ± 2.9	69.6 ± 6.1	0.88	67.4 ± 1.9	68.0 ± 3.1	67.0 ± 6.1	0.98
TGs, mg/dL	122.9 ± 8.8	115.8 ± 11.3	84.8 ± 12.5	0.55	121.2 ± 8.4	114.4 ± 14.3	102.1 ± 11.8	0.60	115.7 ± 9.0	125.3 ± 11.4	125.7 ± 24.5	0.76

CYP, cytochrome P-450; BMI, bone mass index; BMD, bone mineral density; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TGs, triglycerides.
Data are presented as mean ± SE.

total of 248 chromosomes from 124 postmenopausal Japanese women. Figure 1 indicates the location of each SNP analyzed in this study. All SNPs exist within the exon, thus resulting in amino acid substitution.

Although the genotypic distribution of I462V in the *CYP1A1* gene was in Hardy-Weinberg equilibrium, those of R48G and L432V in the *CYP1B1* gene were observed to deviate from Hardy-Weinberg equilibrium. The frequencies of the variant SNP alleles ranged from 19% to 23%. There were no variant alleles in four SNPs (G45D, A463G, A119S, and N453S [*CYP1B1*]) in the population analyzed in this study (Table 1). In addition, no significant differences were observed in the baseline characteristics with any genotypes tested in this study (Table 2). No significant differences were observed in either the baseline characteristics or the response to HT (data not shown).

To test whether these three exon SNPs might be involved in the response to HT, the percentage of changes in the lumbar BMD and the serum lipid profiles after HT were compared according to each genotype of the CYP genes (Table 3). The genotype L432V in the *CYP1B1* gene demonstrated significant associations with lumbar BMD and low-density lipoprotein cholesterol (LDL-C) responses after 12 months of HT. Neither the genotype I462V (*CYP1A1*) nor R48G (*CYP1B1*) demonstrated a significant association with the lumbar BMD or the serum lipid responses. The mean change in the BMD of all women after 12 months of treatment was 2.3 ± 0.5%. Although the absolute value of the BMD did not show any significantly difference among the different genotype groups, the participants with the homozygous (variant) genotype (GG) of L432V showed significantly less BMD change (−3.7 ± 2.4%) than those with the heterozygous (CG; 1.8 ± 1.0%) and homozygous (wild type) (CC; 3.4 ± 0.6%) genotypes. The serum LDL-C level of all women decreased (−13.5 ± 2.7%) after 12 months of treatment. In the women with the heterozygous (CG) and homozygous (CC; wild type) genotypes of L432V, the LDL-C level decreased, whereas that in women with the homozygous (variant) genotype (GG) of L432V inversely increased (11.1 ± 3.5%) after 12 months of treatment.

In the univariate analysis, some factors, other than the L432V polymorphism, significantly influenced the lumbar BMD and LDL-C responses. For example, with older age and a higher baseline BMD, there was less increase in BMD response to HT, and with a higher baseline LDL-C, there was less decrease in LDL-C. Body weight and BMI did not influence those responses to HT.

Finally, the effect of the L432V genotype on the responses of lumbar BMD and LDL-C were maintained after adjustment for the significant variables in the univariate analysis (Table 4). This confirms the independent effect of the L432V polymorphism in the *CYP1B1* gene on the response to HT.

To evaluate the relationship between the L432V SNP and the circulating hormone levels, serum estradiol, LH, and FSH levels after 12 months of HT were compared among the genotypes of L432V. Although the serum levels of estradiol and LH did not show any significant differences, the serum

TABLE 3. *Changes in the lumbar BMD and serum lipids after HT according to the CYP genotypes*

	% change (absolute value)											
	Genotype of I462V (*CYP1A1*)				Genotype of R48G (*CYP1B1*)				Genotype of L432V (*CYP1B1*)			
Variables	AA (n = 78)	AG (n = 42)	GG (n = 4)	*P*	CC (n = 90)	CG (n = 16)	GG (n = 18)	*P*	CC (n = 78)	CG (n = 36)	GG (n = 10)	*P*
L2-4 BMD, g/cm^3	2.4 ± 0.6	2.1 ± 1.2	3.9 ± 1.5	0.833	2.4 ± 0.6	2.6 ± 1.4	1.7 ± 1.2	0.872	3.4 ± 0.6	1.8 ± 1.0	−3.7 ± 2.4	0.002
	(0.78 ± 0.02)	(0.77 ± 0.02)	(0.81 ± 0.09)		(0.78 ± 0.01)	(0.79 ± 0.04)	(0.76 ± 0.04)		(0.78 ± 0.02)	(0.77 ± 0.02)	(0.74 ± 0.06)	
TC, mg/dL	−3.8 ± 2.3	−4.8 ± 1.9	−6.3 ± 6.6	0.9330	−4.5 ± 2.0	−4.0 ± 3.3	−3.1 ± 4.2	0.953	−4.2 ± 1.7	−9.4 ± 3.5	5.5 ± 4.4	0.058
	(212.0 ± 4.6)	(211.3 ± 5.5)	(213.5 ± 5.3)		(212.0 ± 3.5)	(213.4 ± 6.2)	(221.6 ± 8.7)		(210.3 ± 4.5)	(206.1 ± 4.7)	(226.1 ± 11.4)	
LDL-C, mg/dL	−11.0 ± 4.0	−17.4 ± 3.2	−16.6 ± 6.1	0.455	−13.5 ± 3.2	−6.3 ± 6.5	−20.5 ± 7.4	0.302	−15.6 ± 3.8	−18.0 ± 4.2	11.1 ± 3.5	0.002
	(116.8 ± 5.0)	(118.3 ± 3.2)	(114.0 ± 4.4)		(114.6 ± 4.3)	(125.0 ± 5.4)	(124.6 ± 6.9)		(115.6 ± 4.9)	(114.4 ± 4.3)	(140.0 ± 10.9)	
HDL-C, mg/dL	3.0 ± 2.9	8.7 ± 3.5	3.0 ± 3.1	0.408	4.5 ± 2.8	5.3 ± 2.5	7.2 ± 4.1	0.894	4.5 ± 1.9	3.0 ± 6.0	−2.5 ± 4.1	0.827
	(71.3 ± 2.0)	(71.8 ± 2.8)	(78.8 ± 4.0)		(70.8 ± 1.7)	(71.9 ± 3.3)	(75.8 ± 5.2)		(71.5 ± 2.0)	(70.9 ± 2.6)	(68.4 ± 7.4)	
TGs, mg/dL	15.7 ± 6.7	14.5 ± 8.6	38.3 ± 25.8	0.698	19.8 ± 6.5	1.9 ± 14.4	10.7 ± 6.4	0.252	21.2 ± 6.5	5.9 ± 10.3	16.8 ± 13.5	0.357
	(129.6 ± 7.0)	(115.7 ± 10.3)	(111.5 ± 38.3)		(127.9 ± 6.7)	(113.1 ± 11.3)	(115.0 ± 16.3)		(122.1 ± 6.8)	(124.5 ± 11.5)	(137.0 ± 19.9)	

BMD, bone mincral density; HT, hormone therapy, CYP, cytochrome P-450; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol. HDL-C, high-density lipoprotein cholesterol; TGs, triglycerides.
Data are presented as mean ± SE.

level of FSH showed significant differences among the L432V genotypes (Table 5). Compared with the women with the CC genotype (wild type, homozygous), women with the GG genotype (mutant, homozygous) had a significantly higher level of FSH ($P = 0.006$) after 12 months of HT.

DISCUSSION

Variations in the estrogen-metabolizing genes, such as *CYP1A1*, *CYP1B1*, *CYP17*, and *CYP19*, and catechol-*O*-methyltransferase genes have been reported regarding the susceptibility of women to breast cancer, and such variations were also found to influence the clinical course.[13,14] Furthermore, the SNPs of these genes have been evaluated in women using a variety of factors, such as the age at menarche and natural menopause,[15] breast density,[16] and plasma estrogen levels.[17,18]

Both the *CYP1A1* and *CYP1B1* loci appear to play a prominent role within the genes involved in estrogen metabolism. CYP1A1 catalyzes the C2-, C6-, and C15-α hydroxylation, whereas *CYP1B1* catalyzes the C4-hydroxylation of estradiol. Various polymorphic sites of the *CYP1A1* and *CYP1B1* genes have been described on either introns or exons.

In this study, women with a homozygous variant allele of L432V showed significantly poor responses to HT. The genotype frequency distributions of L432V in the *CYP1B1* gene were found to deviate from the Hardy-Weinberg equilibrium because of a variant homozygote excess. This variant in the *CYP1B1* gene is thus possibly an important candidate for an SNP predisposing to the development of either postmenopausal osteopenia or osteoporosis, although the baseline BMD did not significantly differ between the different genotypes in this study.

The catalytic activities of variant enzymes, especially the nucleotide changes in exon 2 (A119S polymorphism) and exon 3 (L432V polymorphism) of the *CYP1B1* gene, have been reported to be two- to fourfold higher than those of wild-type enzymes.[19-22] A significant decrease in the estradiol levels in postmenopausal women with the L432V variant homozygous genotype has been also reported.[18] In this study, significantly higher serum FSH levels during HT in women with an L432V variant genotype were observed, even though there was no significant difference in the serum estradiol level. Although several investigators have shown estradiol to be a predictor of bone loss,[23,24] there is a conflicting report in which there was no significant correlation of estradiol levels with BMD.[25] The peripheral levels of estradiol may not necessarily represent the estradiol levels in target tissues.[26] Thomsen et al[27] reported a strong correlation between the decrease in FSH and the change in BMD, whereas the association between BMD and the estradiol level was less clear. They also reported that women who have a favorable response in BMD during HT also tend to show a favorable change in the lipid profile, and this association is most likely driven by a common response of FSH to exogenous estrogen therapy. Therefore, the L432V variant that corresponds to the hyperactivity of *CYP1B1* accelerates estradiol metabolism, thus leading to higher serum FSH levels and thus may possibly affect the response to HT regarding the lumbar BMD and serum lipid profiles.

There are some limitations to our study. Gonadotropins are known to be secreted in an episodic fashion. The pulse amplitude of FSH in postmenopausal women with HT has been reported to be 5.7 ± 1.0 mIU/mL. Therefore, the validity of the gonadotropin determinations based on a single blood measurement may be questioned. In addition, the number of the L432V variants in this study was limited. Additional studies are therefore necessary to clarify the precise mechanisms by which the *CYP1B1* polymorphisms modulate the responsiveness of BMD and LDL-C to HT.

TABLE 4. *Baseline variables as predictors of the percent change in the lumbar BMD and serum LDL-C after HT: multivariate regression analysis*

Variables	Correlation coefficient *r*	*P*
BMD		
Age	0.130	0.107
Baseline BMD	−0.416	<0.001
L432V (CYP1B1) genotype	0.273	<0.001
LDL-C		
Baseline LDL-C	−0.501	<0.001
L432V (CYP1B1) genotype	0.182	0.039

BMD, bone mineral density; LDL-C, low-density lipoprotein cholesterol; HT, hormone therapy.

TABLE 5. *Serum hormone levels at 12 months after HT according to the genotype of L432V in the CYP1B1 gene*

	Genotype			
	CC (n = 20)	CG (n = 20)	GG (n = 10)	*P*
Estradiol, pg/mL	71.3 ± 7.3	74.3 ± 14.3	69.9 ± 16.8	0.971
LH, mIU/mL	11.2 ± 2.6	15.5 ± 3.2	16.2 ± 6.2	0.560
FSH, mIU/mL	9.4 ± 1.1	15.7 ± 3.3	24.1 ± 6.4	0.021

HT, hormone therapy; LH, luteinizing hormone; FSH, follicle-stimulating hormone.
Data are presented as mean ± SE. Controlling for age, date of blood draw, time of blood draw, fasting status, body mass index, and laboratory batch.

CONCLUSIONS

In summary, our genetic analyses of the genes *CYP1A1* and *CYP1B1* suggest that the L432V SNP in the *CYP1B1* gene might act as a marker of the drug response. An analysis of the *CYP1B1* gene SNPs might therefore prove to be useful in appropriately selecting HT for the management of either osteopenia or hyperlipidemia in Japanese postmenopausal women.

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Pseudo outbreak of *Burkholderia cepacia* in vaginal cultures and intervention by hospital infection control team

Madam,

Burkholderia cepacia has been increasingly recognised as a nosocomial pathogen and has been cultured from clinical devices in the hospital environment. Since it is highly resistant to disinfectants such as chlorhexidine gluconate and benzalkonium chloride, it may act as an opportunistic pathogen especially in immunocompromised patients.[1,2] We describe a nosocomial outbreak of *B. cepacia* in vaginal secretions that occurred in patients attending our hospital.

Niigata University Medical and Dental Hospital is a tertiary care university teaching hospital with 810 beds and tertiary care in Niigata, Japan. The obstetric ward has 22 beds, and the gynaecology ward has 35 beds. There are about 30 obstetricians and gynaecologists in our hospital, and about 100 outpatients come to the outpatient ward per day. Each week, trends in bacterial isolation as well as all drug-resistant bacteria detected in our hospital are discussed by the infection control team. In April and May 2003, a report from the medical laboratory division showed that the detection frequency of *B. cepacia* was increasing in obstetrics and gynaecology-related vaginal specimens. In order to decide whether these results constituted an outbreak, the monthly positive rate, mean positive rate and SD of *B. cepacia* in vaginal cultures for the past two years were investigated retrospectively. On the basis of the medical laboratory division records from January 2001 to December 2002, 178 episodes of *B. cepacia* vaginitis occurred among 2499 patients. The highest monthly incidence of vaginal cultures positive for *B. cepacia* was seen in September 2001 (14.2%) and the lowest was seen in January 2002 (0%). The average positivity rate was 7.1% and the SD was 4.7%. An outbreak was defined as a positive rate +2 SD above

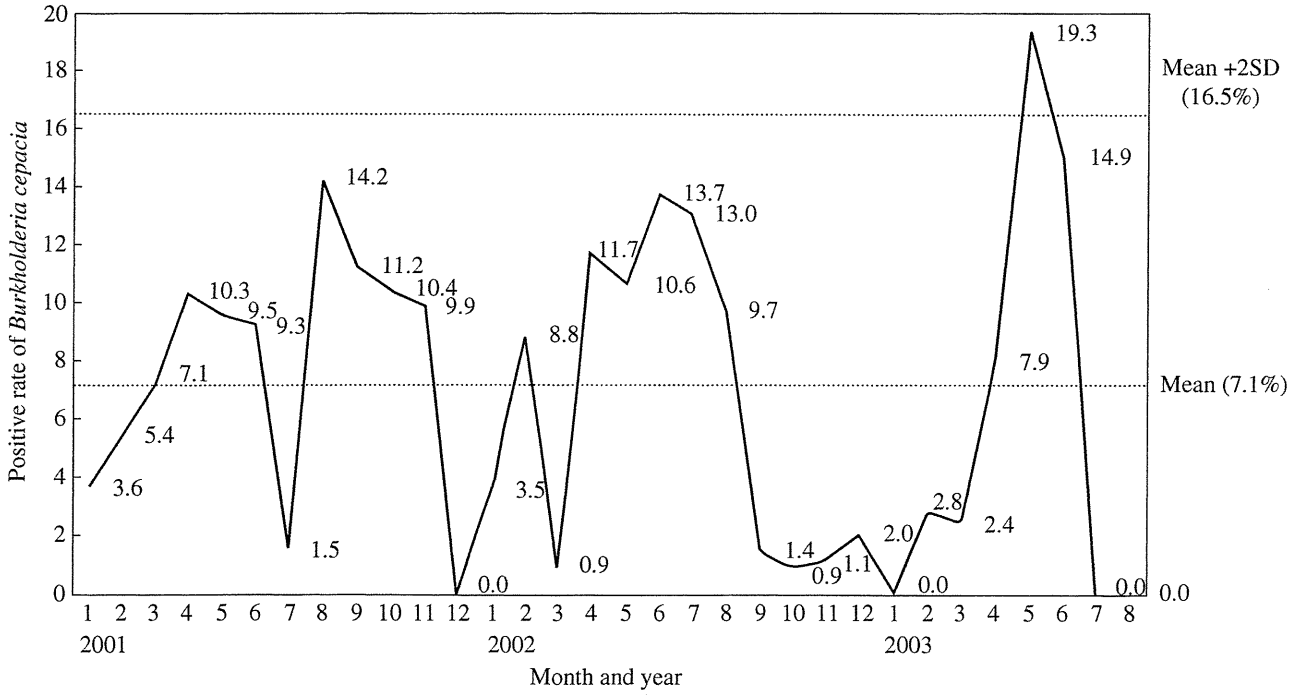

Figure 1. *Burkholderia cepacia*-positive rate in the Niigata University Medical and Dental Hospital. The range of the incidence of *B. cepacia* positives for the years 2001–2002 was 0–14.2%, with an average of 7.1%. The SD was 4.7% so that the incidence level of an outbreak would be 16.5% (the average rate + 2 SD). The positive rate increased from April and was above the threshold in May 2003. It was judged that there was possibility of an outbreak.

the average of the past two years (16.5%). In May 2003, *B. cepacia* was detected in 17 (19.3%) of 88 patients (Figure 1); therefore we concluded that an outbreak had occurred. In the patients in whom *B. cepacia* was detected, however, subjective symptoms such as fever, an increase of discharge or objective symptoms such as increases in C-reactive protein or white blood cells were not detected.

Environmental research was carried out to investigate the common source of infection. There were ten irrigators at outpatient and inpatient wards in our hospital. The benzalkonium chloride solution in all the irrigators was cultured. Next, undiluted solutions of benzalkonium chloride and the distilled water used for preparation were cultured. A total of nine environmental samples were collected. *B. cepacia* was cultured from all irrigators whereas *Pseudomonas aeruginosa* was not cultured from any. *B. cepacia* was also detected in the unopened 0.02% benzalkonium chloride liquid prepared in our hospital. In addition, *B. cepacia* was not grown from undiluted benzalkonium chloride and distilled water manufactured in our hospital.

We stopped using irrigators immediately. When patients needed vagina disinfection, we used commercially available 0.25% inverted soap. Examining hands or vaginal speculi were moistened with saline when we performed pelvic or speculum examinations. By stopping use of the irrigators and using commercial 0.025% benzalkonium chloride, no more *B. cepacia* was found in patient specimens.

We had used exclusive containers for the 0.02% benzalkonium chloride prepared in our hospital. We washed them with heat-disinfected distilled water after returning from each ward before filling, but we did not dry the containers each time. The benzalkonium chloride solution in all irrigators in each ward had been exchanged once a week. The exchange method was such that fresh solution was poured into the irrigator after discarding old solution and rinsing gently. Therefore, we suspect that the 0.02% benzalkonium chloride prepared in our hospital for pelvic examinations was the common source of *B. cepacia* infection. We considered that *B. cepacia* had colonised the container and increased within containers or irrigators, and that the patient's vagina had been exposed to *B. cepacia* at the time of an internal examination. After use of irrigators had been stopped at each ward and commercial benzalkonium chloride had been adopted, *B. cepacia* was not seen. We use saline for internal examinations and there are no clinical problems without using irrigators.

We should not overestimate the ability of a disinfectant to prevent nosocomial infection, and prohibition of any additional antiseptics should be mandatory. Moreover, as a prompt intervention measure against infection at the time of the outbreak was useful for preventing further cases, it seems that surveillance of vaginal culture results should be considered for early detection of an outbreak.

Conflict of interest statement
None declared.

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Are open box gloves clean enough to perform vaginal examinations?☆

Madam,

In most obstetric offices in the USA, vaginal examinations are performed with single-use open box gloves. Once that same patient is in a labour ward, vaginal examinations are performed with single-packaged sterile gloves. As the fiscal and environmental costs of modern healthcare are reviewed, interest in using open box gloves in the inpatient setting has developed. Ignac Semmelweis demonstrated that the best-known route of infection in hospitals is via direct contact from healthcare workers.[1] Hand washing is fundamental to infection control. Compliance with mandatory institutional hand-washing guidelines is moderate at best.[2–4] This, along with the generalised fear of infection in labour, has led to routine sterile glove use. A review of the medical literature found no data regarding glove choice for women in labour. In this pilot study, our aim was to evaluate a sample of open box gloves found in labour and delivery as potential fomites. These gloves are used by staff for patient care outside of vaginal examinations. We hypothesised that open box gloves would not be contaminated with major pathogens.

To test this hypothesis, a representative glove was sampled from in-use boxes from ten labour and delivery rooms. The gloves were removed with sterile forceps and placed in a sterile specimen bag. Sterile broth medium was then poured into the bag and the fluid was agitated around all surfaces of the glove. The sampled glove was then removed and the fluid incubated. A packaged sterile glove was sampled in a similar fashion as a negative control. As a positive control, each labour and delivery room that had a glove sampled also had aerobic cultures obtained from the room computer keyboard and mouse using a sterile swab, that was immersed in transport medium, then plated out in the laboratory. Following 48 h incubation, broth specimens were plated on colistin nalidixic acid and

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Increased pulsatility of the ductus venosus blood velocity in the first trimester is associated with the delivery of small for gestational age or low birth weight infants

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Abstract

Aim: To determine the relationship between ultrasound findings during the first trimester and obstetrical outcomes.

Methods: A retrospective cohort study was carried out on 516 women who underwent ultrasound examination between 12 and 14 gestational weeks.

Results: The reduced crown-rump length, biparietal diameter, femur length (Z-score < -1 and -1.5) and increased umbilical artery pulsatility index (>90th and 95th percentile) were not associated with poor obstetrical outcomes. A ductus venosus pulsatility index greater than the 90th percentile was associated with aneuploidy, small for gestational age and low birth weight infants.

Conclusion: Increased pulsatility in the ductus venosus is associated with increased risk for chromosomal abnormality in early pregnancy. Here we show that increased pulsatility is also linked to fetal growth restriction.

Key words: ductus venosus, fetal growth restriction, first trimester, low birth weight, small for gestational age.

Introduction

Ultrasound examinations during the first trimester have had several important clinical consequences, such as the diagnosis of missed miscarriage, accurate dating of pregnancy, early diagnosis of major anomalies and screening for trisomy 21 and other forms of aneuploidy.[1] Recently, some reports have suggested that fetal growth restriction may occur in the first trimester and are associated with the delivery of small for gestational age (SGA) infants.[2–4] Increased nuchal translucency (NT) and abnormal ductus venosus (DV) flow in the first trimester have been reported to be associated with aneuploidy, cardiac defect and other fetal abnormalities[1,5–10] Therefore, ultrasound examination during the first trimester has been increasingly regarded as more important than before. In our hospital, all of the pregnant subjects had undergone ultrasound examination as screening between 12 and 14 gestational weeks (GW). In this study, we analyzed the relationship between ultrasound findings and obstetrical outcomes.

Methods

This retrospective cohort study was approved by the Ethics Committee of Niigata University. All singleton pregnant women who were under the care of Niigata University Medical and Dental Hospital between December 2003 and September 2006 ($n = 614$) underwent ultrasound examination between 12 and 14 GW (first trimester scan). The study population was a mixture of low-risk and high-risk pregnant women, including referrals.

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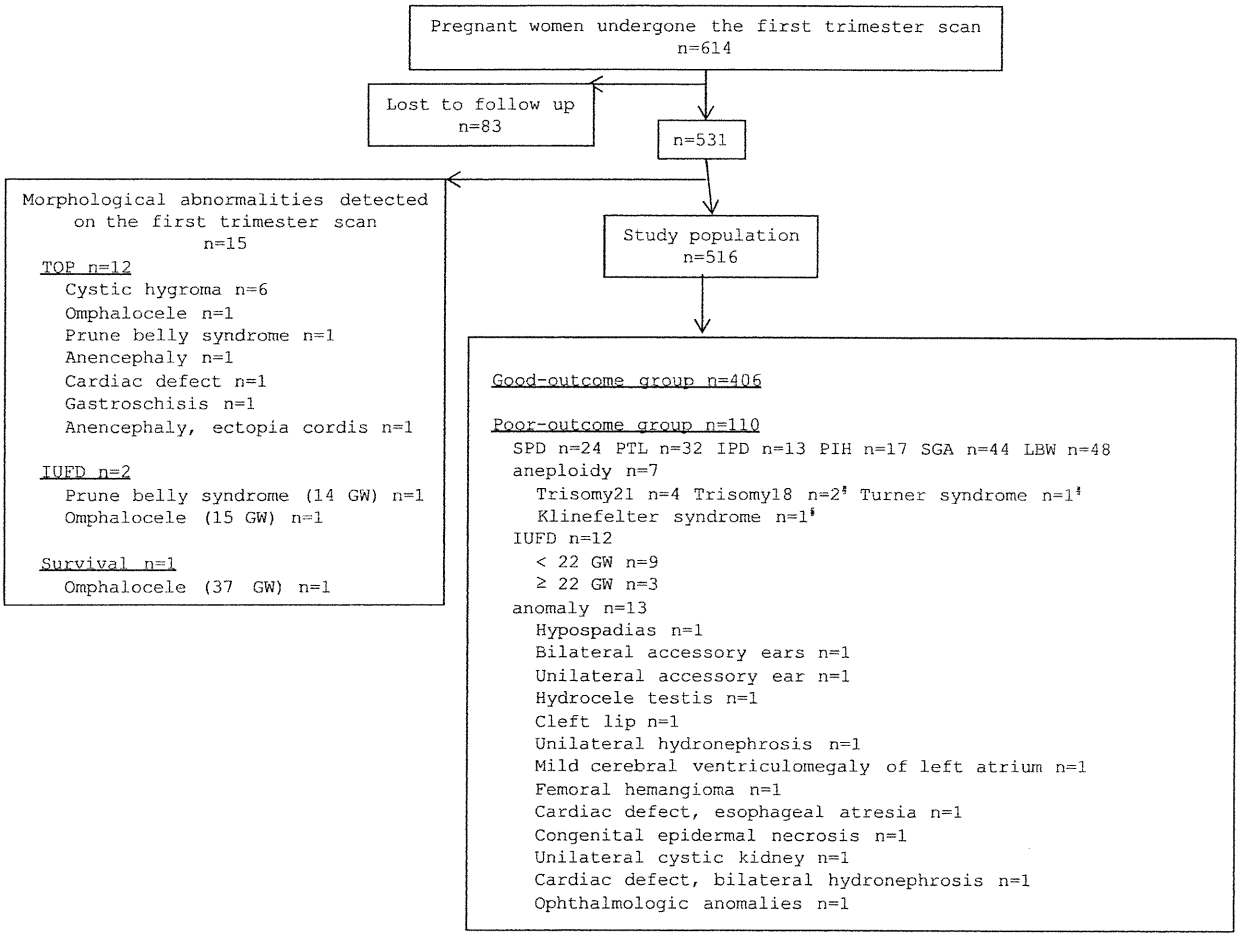

Figure 1 Study design. GW, gestational week; IPD, iatrogenic preterm delivery; IUFD; intrauterine fetal death; LBW, low birth weight; PIH, pregnancy-induced hypertension; PTL; preterm labor; SGA, small for gestational age; SPD, spontaneous preterm delivery; TOP; termination of pregnancy; #, cases resulting in TOP ($n = 4$).

We gathered data on gestational age at delivery, birth weight, fetal anomalies and aneuploidy, mode of delivery, whether occurrence of preterm labor (PTL) had required any treatment, and pregnancy-induced hypertension (PIH) from the hospitals where the women had delivered (our hospital and other hospitals). A normal infant was defined as one who was born after 37 GW with an appropriate birth weight (≤90th percentile, ≥10th percentile) and no congenital abnormalities. Those who had not been suspected of having anomalies or aneuploidy prenatally were judged by physical examinations and observed for approximately one month as to whether they were normal or not. Eighty-three women were lost to follow-up and the data of 531 patients could be obtained. Of those 531 women, morphological abnormalities (excluding increased NT), were detected in 15 fetuses during the first trimester scan. These 15 patients were excluded from the study; therefore, 516 patients were finally enrolled in the study (Fig. 1) and their characteristics are presented in Table 1. Poor outcome was defined as the occurrence of spontaneous preterm delivery (SPD), PTL, iatrogenic preterm delivery (IPD) or PIH, and delivery of an infant with SGA, low birth weight (LBW), aneuploidy or anomaly. SPD is defined as delivery at less than 37 GW as a result of PTL or preterm rupture of membranes. IPD is defined as induced preterm delivery at less than 37 GW as a result of intrauterine fetal growth restriction (IUGR), intrauterine fetal death (IUFD), non-reassuring fetal status or maternal conditions such as PIH, placenta previa and placental abruption. PIH was diagnosed where there

Table 1 Characteristics of the study population ($n = 516$)

	n	%	Median	Range
Maternal age at delivery (years)			32	19–45
Parity				
Nulliparous	270	52.3		
Parous	246	47.7		
Gestational days at scan			95	84–104
Abortion†	13	2.5		
Delivery‡				
Emergency cesarean section	52	10.3		
Elective cesarean section	50	9.9		
Vaginal delivery	401	79.7		
Birth weight (g)‡			3070	455–4302
Non-obstetrical complications				
Gastrointestinal disorders	4	0.8		
Connective tissue disorders	9	1.7		
Heart diseases	1	0.2		
Cerebrovascular disorders	1	0.2		
Hematological disorders	4	0.8		
Chronic hypertension	6	1.2		
Asthma	17	3.3		
Thyroid disorders	15	2.9		
Diabetes mellitus	11	2.1		
Gynecologic disorders	72	14.0		
Psychiatric disorders	8	1.6		
Epilepsy	8	1.6		
Renal disorders	9	1.7		
Obstetrical complications				
Rhesus blood group incompatibility	5	1.0		
Gestational diabetes mellitus	4	0.8		
Cervical incompetency	2	0.4		
Placental abruption	2	0.4		
Placenta previa	1	0.2		

†Abortion means termination of pregnancy and intrauterine fetal death before 22 gestational weeks; ‡Cases resulting in abortion were excluded.

was systolic blood pressure higher than 140 mmHg and/or diastolic blood pressure higher than 90 mmHg after 20 GW. SGA is defined as birth weight less than the 10th percentile of the Japanese standard. LBW is defined as birth weight less than 2500 g. Amniocentesis for fetal karyotyping was offered to the pregnant women who would be at least 35 years old on the expected day of delivery, for those who had previously carried a fetus with aneuploidy, had a chromosomal translocation, had a partner with chromosomal translocation, had a fetus with NT greater than 3 mm or who had major structural defects identified by ultrasound examination. Neonatal karyotyping was offered to infants who had multiple structural defects or the appearance of aneuploidy.

The gestational age was determined by the last menstrual period and ascertained by a crown-rump length (CRL) of between approximately 14 mm and 41 mm. The gestational age was altered by CRL measurement when estimated to be more than 7 days earlier than the date of the last menstrual period. The ultrasound examination routinely included measurement of the CRL, biparietal diameter (BPD), femur length (FL), NT, umbilical artery (UA) pulsatility index (PI) and ductus venosus pulsatility (DVPI). Umbilical artery pulsatility index (UAPI) = (peak systolic velocity – end-diastolic velocity)/time-averaged maximum velocity, DVPI = (peak systolic velocity – velocity during atrial contraction)/time-averaged maximum velocity. The first trimester scans were performed transabdominally using a Voluson 730 Expert (GE Medical System, Milwaukee, WI, USA). NT measurement was performed according to the method described by Nicolaides.[1] An NT value of ≥3 mm was considered abnormal. The DV can be identified, preferably near the midsagittal plane where it leaves the portal sinus to join the inferior vena cava. The direction of flow was confirmed by color Doppler imaging.

The pulse Doppler gate was placed in the inlet of the DV.[11] The UA was identified in the amniotic fluid and the pulse Doppler gate was placed in the straightest portion. The insonation angle was always <60°. Pulsed Doppler signals were collected by a sample volume of 2.5–5 mm placed above the origin of the DV and the UA.

The reference ranges of each parameter between 12 and 14 GW were determined by the measurements values obtained from women who did not suffer PIH and delivered a normal infant after 37 GW. The mean values and standard deviations (SD) of CRL, BPD and FL at different gestational days were regressed using a simple linear equation. As references for DVPI and UAPI, the 5th, 10th, median, 90th and 95th percentiles in the different GW were calculated. To facilitate comparisons, the values of CRL, BPD and FL were converted to Z-scores according to the GW at the time of the scans. The Z-score was determined by the following formula: (XGD – MGD)/SDGD, where XGD is the measured value on a known gestational day, MGD is the mean value and SDGD is the SD obtained from the reference equations. The associations of these parameters with the obstetrical outcome were analyzed by Fisher's exact test and Kruskal–Wallis test. $P < 0.05$ was considered statistically significant. Cases resulting in termination of pregnancy (TOP) were excluded in analyses of SPD, PTL, IPD, PIH, SGA and LBW. Cases with fetal anomaly, fetal aneuploidy and IUFD were excluded in the analyses of SGA and LBW. Cases with IUFD before 22 GW were excluded in analyses of SPD, PTL, IPD and PIH. Cases with IUFD without sufficient information about the macroscopic findings and karyotype were excluded in analyses of fetal anomaly and fetal aneuploidy.

Results

Reference values between 12 GW and 14 GW

We determined the reference values of CRL, BPD, FL, UAPI and DVPI. The measurement values of CRL ($n = 339$), BPD ($n = 359$), FL ($n = 344$), UAPI ($n = 333$) and DVPI ($n = 277$) were obtained from women who did not suffer PIH and delivered a normal infant after 37 GW. Regression analysis demonstrated a significant positive correlation between the fetal biometry (CRL, BPD and FL) and gestational day (GD). The mean value and the SD of each biometry between 12 and 14 weeks of gestation were given by the following linear equations.

$$\text{CRL (mm)} = 1.501 \times \text{GD} - 69.780 \quad (R2 = 0.9788,\ P < 0.0001)$$

$$\text{SD-CRL (mm)} = 0.03390 \times \text{GD} + 3.095 \quad (R2 = 0.03541)$$

$$\text{BPD (mm)} = 0.5301 \times \text{GD} - 25.59 \quad (R2 = 0.9767,\ P < 0.0001)$$

$$\text{SD-BPD (mm)} = 0.04403 \times \text{GD} - 2.457 \quad (R2 = 0.4765)$$

$$\text{FL (mm)} = 0.4543 \times \text{GD} - 32.45 \quad (R2 = 0.963,\ P < 0.0001)$$

$$\text{SD-FL (mm)} = 0.05545 \times \text{GD} - 3.641 \quad (R2 = 0.2727)$$

The individual measurement values of UAPI and DVPI were plotted on the GD shown in Figure 2. We compared the data lumped for each GW by using the Kruskal–Wallis test and found that the values of UAPI and DVPI were significantly different depending on GW when they were measured. The 5th, 10th, median, 90th and 95th percentiles of the UAPI and DVPI in each GW are shown in Table 2. Using these reference ranges, the relationship of the biometry and pulse Doppler findings to the obstetrical outcomes was analyzed as follows.

Relationship between ultrasound findings and obstetrical outcomes

The results of the ultrasound findings and karyotypes of the poor-outcome group and good-outcome group are shown in Table 3.

Table 4 shows the effects of small CRL, BPD and FL (Z-score < –1 and –1.5) on the occurrence of SPD, PTL, IPD, PIH, fetal aneuploidy, fetal anomalies and delivery of SGA and LBW infants. No significant relationships were observed between small biometry and poor obstetrical outcomes. As shown in Table 5, an NT ≥ 3 mm was significantly associated with fetal aneuploidy (OR52.9, 95%CI 11.55–241.1). An increased UAPI (>90th and 95th percentiles) had no significant effect on the outcomes, and an increased DVPI (>90th and 95th percentiles) was a risk factor for SGA, LBW and fetal aneuploidy. Three of five fetuses with aneuploidy had a DVPI greater than the 90th percentile. Of three fetuses with aneuploidy and increased DVPI, two with trisomy 18 had an abnormal NT, but one with trisomy 21 did not have an abnormal NT (data not shown).

Discussion

It is considered that fetal growth in early pregnancy is minimally affected by pathological disorders. However, recent reports have suggested that fetal

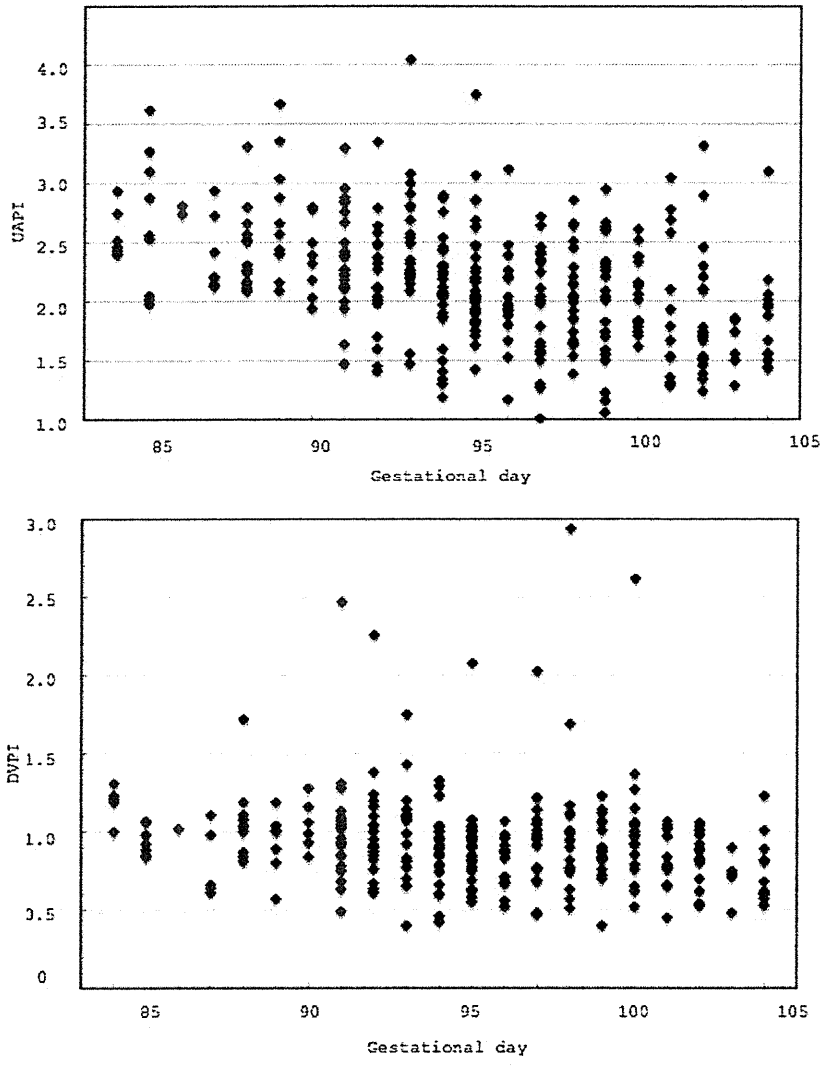

Figure 2 Relationship between gestation day and umbilical artery pulsatility index (UAPI) or ductus venosus pulsatility (DVPI). The measurement values of UAPI and DVPI of normal fetuses are plotted individually on each gestational day.

Table 2 Reference ranges of umbilical artery pulsatility index (UAPI) and ductus venosus pulsatility (DVPI)

	5th %tile	10th %tile	Median	90th %tile	95th %tile
UAPI					
12 GW	2.03	2.09	2.5	3.08	3.33
13 GW	1.41	1.53	2.19	2.84	2.96
14 GW	1.31	1.44	1.87	2.63	2.8
DVPI					
12 GW	0.64	0.80	1.00	1.21	1.28
13 GW	0.54	0.63	0.91	1.22	1.34
14 GW	0.53	0.59	0.85	1.21	1.23

%tile, percentile; GW, gestational weeks.

Table 3 Results of ultrasound findings and karyotypes of the poor-outcome group and good-outcome group

		Good-outcome group ($n = 406$)			Poor-outcome group ($n = 110$)		
		Mean or median	SD or IQR	n	Mean or median	SD or IQR	n
CRL z score		0.04†	0.95‡	381	−0.12†	1.03‡	103
BPD z score		0.04†	1.00‡	403	0.11†	1.05‡	109
FL z score		−0.05†	1.05‡	388	−0.18†	1.10‡	103
UAPI							
	12w	2.50§	2.18–2.79¶	63	2.46§	2.20–2.71¶	20
	13w	2.20§	1.92–2.48¶	182	2.22§	1.84–2.47¶	49
	14w	1.86§	1.56–2.21¶	129	1.76§	1.51–2.25¶	30
DVPI							
	12w	1.00§	0.86–1.09¶	48	0.94§	0.90–1.50¶	14
	13w	0.91§	0.76–1.04¶	152	0.97§	0.84–1.17¶	40
	14w	0.84§	0.73–1.01¶	107	0.71§	0.71–1.06¶	25
NT ≥ 3mm (%)				12/397 (3.0%)			11/108 (10.2%)
Fetal karyotyping				10			9
	Normal			10			3
	Abnormal			0			6
Neonatal karyotyping				0			2
	Normal			0			0
	Abnormal			0			2

†Mean; ‡SD; §median; ¶IQR. BPD, biparietal diameter; CRL, crown-rump length; DV, ductus venosus; DVPI, ductus venosus pulsatility; FL, femur length; IQR, interquartile range; NT, nuchal translucency; PI, pulsatility index; SD, standard deviation; UA, umbilical artery.

growth restriction may be present as early as in the first trimester and is associated with poor obstetrical outcomes.[2–4] In this study, reduced CRL, BPD and FL between 12 and 14 GW had no significant association with delivery of SGA and LBW infants or other poor obstetrical outcomes. It may be impossible to detect statistical differences because this study population of our research was smaller than those of earlier reports.

A DVPI greater than the 90th percentile in the first trimester was significantly associated with delivery of SGA and LBW infants. The DV is a venous shunt between the intra-abdominal umbilical vein and the inferior vena cava. An increase in the DV shunting rate is a general adaptation mechanism in the presence of placental insufficiency to ensure an adequate supply of oxygen and glucose to vitally important organs, such as the brain and heart, but it also results in a reduction of hepatic blood supply.[12–15] An increased DVPI has commonly been found in IUGR and SGA cases in the second or third trimesters. It has been reported that an increased DVPI was observed several days prior to delivery in IUGR fetuses[16–19] and that Doppler examination of DV was useful to predict fetal compromise, morbidity and mortality and to determine optimal timing of intervention.[16–18,20,21] Our results suggest the possibility that a change in DV flow may occur as early as in the first trimester in some growth-restricted fetuses. There are, however, few reports on the relationship between abnormal DV flow in the first trimester and the risk of SGA or LBW infants. Oh *et al.* reported that there were no differences in the ratio of IUGR and birth weight between fetuses with abnormal DV flow and a control group.[22] Oh *et al.* defined abnormal DV flow as absent or reverse flow during atrial contraction,[22] which have often been used as a DV parameter in studies on associations with aneuploidy and cardiac defects,[5–10] while we look at the DVPI value, which can evaluate the DV flow velocity quantitatively. The degree of abnormal DV flow observed in growth-restricted fetuses may be so mild that the association can not be proved using absent or reverse flow during atrial contraction as a parameter. A DVPI increase up to a CRL of 63 mm (12 weeks and 6 days) and the cause thereof is considered as the absence of trophoblastic migration in early pregnancy. In the trophoblastic migration, placental vascular resistance decreases and, as a result, the DVPI decreases.[23] Inadequate trophoblastic migration may be the cause of the increased DVPI observed in growth-restricted fetuses between 12 and 14 GW.

Table 4 Association between perinatal outcome and biometry

Poor outcome	*n*§	*n*¶	Positive† negative‡ CRL Z score < −1.0		OR	95%CI			*P-value*
SPD	23	447	5/75	18/395	1.50	0.54	–	4.16	0.3909
PTL	28	442	5/75	23/372	1.16	0.42	–	3.14	0.7899
IPD	13	457	2/75	11/395	0.96	0.21	–	4.41	1.0000
SGA	40	412	8/72	32/380	1.36	0.60	–	3.09	0.4961
LBW	47	405	9/72	38/380	1.29	0.59	–	2.79	0.5285
PIH	16	454	2/75	14/395	0.75	0.17	–	3.35	1.0000
Aneuploidy	8	467	2/77	6/398	1.74	0.34	–	8.80	0.6216
Anomaly	12	462	2/77	10/397	1.03	0.22	–	4.81	1.0000
			CRL Z score < −1.5						
SPD	23	447	2/23	21/447	1.93	0.42	–	8.79	0.3121
PTL	28	442	0/23	28/447	0.31	0.02	–	5.29	0.3854
IPD	13	457	2/23	11/447	3.78	0.79	–	18.13	0.1287
SGA	40	412	4/31	36/431	2.58	0.82	–	8.09	0.1043
LBW	47	405	3/21	44/431	1.47	0.42	–	5.18	0.4702
PIH	16	454	2/23	14/447	2.95	0.63	–	13.81	0.1813
Aneuploidy	8	467	0/23	8/452	1.11	0.06	–	19.88	1.0000
Anomaly	12	462	1/23	11/451	1.82	0.22	–	14.73	0.4534
			BPD Z score < −1.0						
SPD	24	476	2/77	22/423	0.49	0.11	–	2.11	0.5595
PTL	32	468	5/77	27/423	1.02	0.38	–	2.73	1.0000
IPD	13	487	2/77	11/423	1.00	0.22	–	4.60	1.0000
SGA	44	437	8/76	36/405	1.21	0.54	–	2.71	0.6646
LBW	48	433	7/76	41/405	0.90	0.39	–	2.09	1.0000
PIH	17	483	4/77	13/423	1.73	0.55	–	5.45	0.3132
Aneuploidy	8	497	1/78	7/427	0.78	0.09	–	6.43	1.0000
Anomaly	13	491	0/78	13/426	0.20	0.01	–	3.32	0.2354
			BPD Z score < −1.5						
SPD	24	476	0/31	24/469	0.29	0.02	–	4.86	0.3874
PTL	32	468	3/31	29/469	1.63	0.47	–	5.67	0.4384
IPD	13	487	1/31	12/469	1.27	0.16	–	10.10	0.5694
SGA	44	437	2/30	42/451	0.70	0.16	–	3.02	1.0000
LBW	48	433	3/30	45/451	1.00	0.29	–	3.44	1.0000
PIH	17	483	3/31	14/469	3.48	0.94	–	12.83	0.0812
Aneuploidy	8	497	1/32	7/473	2.15	0.26	–	18.02	0.4099
Anomaly	13	491	0/32	13/472	0.52	0.03	–	9.01	1.0000
			FL Z score < −1.0						
SPD	23	478	4/84	19/417	1.05	0.35	–	3.16	1.0000
PTL	29	430	7/84	22/375	1.46	0.60	–	3.54	0.4546
IPD	12	447	3/84	9/375	1.51	0.40	–	5.69	0.4666
SGA	43	401	9/82	34/362	1.19	0.55	–	2.59	0.6796
LBW	46	398	9/82	37/362	1.08	0.50	–	2.34	0.8415
PIH	17	442	4/84	13/375	1.39	0.44	–	4.38	0.5289
Aneuploidy	7	456	2/85	5/378	1.80	0.34	–	9.43	0.6173
Anomaly	10	452	1/85	9/377	0.49	0.06	–	3.90	0.6971
			FL Z score < −1.5						
SPD	23	478	2/40	21/461	1.10	0.25	–	4.88	0.7045
PTL	29	430	2/40	27/419	0.76	0.17	–	3.34	1.0000
IPD	12	447	2/40	10/419	2.15	0.45	–	10.19	0.2814
SGA	43	401	5/39	38/405	1.42	0.52	–	3.85	0.5670
LBW	46	398	5/39	41/405	1.31	0.48	–	3.52	0.5820
PIH	17	442	2/40	15/419	1.42	0.31	–	6.44	0.6521
Aneuploidy	7	456	1/41	6/422	1.73	0.20	–	14.77	0.4798
Anomaly	10	452	1/41	9/421	1.14	0.14	–	9.27	0.6090

P-values were determined by Fisher's exact test. †Number of the poor outcomes with the positive finding/number of total study population with the positive findings; ‡number of the poor outcomes with the negative finding/number of total study population with the negative findings; §number of cases with the poor outcome with data; ¶number of cases without the poor outcomes with data. BPD, biparietal diameter; CI, confidence interval; CRL, crown-rump length; FL, femur length; IPD, iatrogenic preterm delivery; LBW, low birth weight; OR, odds ratio; PIH, pregnancy-induced hypertension; PTL, preterm labor; SGA, small for gestational age; SPD, spontaneous preterm delivery.

Table 5 Association between perinatal outcome and Doppler study or nuchal translucency (NT)

			Positive† negative‡					
	n§	*n*¶	UAPI > 90th %tile	OR	95%CI			*P-value*
SPD	23	442	0/42	23/423	0.20	0.01	–	3.36	0.2509
PTL	29	436	3/42	26/423	1.18	0.34	–	4.06	0.7378
IPD	12	453	0/42	12/423	0.39	0.02	–	6.66	0.6128
SGA	40	407	4/40	36/407	1.15	0.39	–	3.40	0.7716
LBW	46	402	3/40	43/408	0.69	0.20	–	2.33	0.7849
PIH	16	449	1/42	15/423	0.66	0.09	–	5.15	1.0000
Aneuploidy	6	463	1/43	5/426	2.01	0.23	–	17.57	0.4402
Anomaly	13	455	2/42	11/426	1.89	0.40	–	8.81	0.3284
				UAPI > 95th %tile					
SPD	23	442	0/24	23/441	0.36	0.02	–	6.17	0.6223
PTL	29	436	3/24	26/441	2.28	0.64	–	8.15	0.1822
IPD	12	453	0/24	12/441	0.70	0.04	–	12.20	1.0000
SGA	40	407	4/23	36/424	2.27	0.73	–	7.03	0.1398
LBW	46	402	3/23	43/425	1.33	0.38	–	4.67	0.7200
PIH	16	449	0/24	16/441	0.53	0.03	–	9.04	1.0000
Aneuploidy	6	463	1/25	5/444	3.66	0.41	–	32.57	0.2814
Anomaly	13	455	1/24	12/444	1.57	0.19	–	12.57	0.5002
				DVPI > 90th %tile					
SPD	21	360	3/44	18/337	1.30	0.37	–	4.60	0.7221
PTL	29	352	5/44	24/337	1.67	0.60	–	4.64	0.3586
IPD	10	371	1/44	9/337	0.85	0.10	–	6.86	1.0000
SGA	30	337	7/41	23/326	2.71	1.08	–	6.79	0.0613
LBW	36	331	8/41	28/326	2.58	1.09	–	6.12	0.0450
PIH	11	370	2/44	9/337	1.74	0.36	–	8.31	0.3692
Aneuploidy	5	379	3/46	2/338	11.72	1.90	–	72.17	0.0136
Anomaly	10	374	2/46	8/338	1.88	0.39	–	9.12	0.3413
				DVPI > 95th %tile					
SPD	21	360	1/29	20/352	0.59	0.08	–	4.59	1.0000
PTL	29	352	4/29	25/352	2.09	0.68	–	6.49	0.2604
IPD	10	371	1/29	9/352	1.36	0.17	–	11.14	0.5514
SGA	30	337	7/26	23/341	5.09	1.94	–	13.37	0.0026
LBW	36	331	7/26	29/341	3.96	1.54	–	10.21	0.0080
PIH	11	370	2/29	9/352	2.82	0.58	–	13.73	0.2007
Aneuploidy	5	379	2/30	3/354	8.36	1.34	–	52.13	0.0509
Anomaly	10	374	2/30	8/354	3.09	0.63	–	15.26	0.1795
				NT ≥ 3 mm					
SPD	24	470	0/16	24/478	0.56	0.03	–	9.66	1.0000
PTL	31	463	0/16	31/478	0.43	0.03	–	7.35	0.6139
IPD	13	481	1/16	12/478	2.59	0.32	–	21.23	0.3517
SGA	43	432	0/12	43/463	0.39	0.02	–	6.65	0.6130
LBW	48	427	0/12	48/463	0.34	0.02	–	5.88	0.6205
PIH	17	477	0/16	17/478	0.80	0.05	–	13.88	1.0000
Aneuploidy	8	491	5/20	3/479	52.89	11.55	–	242.10	<0.0001
Anomaly	13	485	2/19	11/479	5.01	1.03	–	24.37	0.0837

P-values were determined by Fisher's exact test. †Number of the poor outcomes with the positive finding/number of total study population with the positive findings; ‡number of the poor outcomes with the negative finding/number of total study population with the negative findings; §number of cases with the poor outcome with data; ¶number of cases without the poor outcomes with data. %tile, percentile; CI, confidence interval; DV, ductus venosus; IPD, iatrogenic preterm delivery; LBW, low birth weight; OR, odds ratio; PI, pulsatility index; PIH, pregnancy-induced hypertension; PTL, preterm labor; SGA, small for gestational age; SPD, spontaneous preterm delivery; UA, umbilical artery, UAPI, umbilical artery pulsatility index.

In this study, abnormal NT and high DVPI were significantly associated with fetal aneuploidy and many studies have already suggested an association between aneuploidy and abnormal DV flow.[1,5–10] In two reports, in which the DVPI was used as the parameter, the 95th percentile was used as the cutoff value.[24,25] Of our study patients, there was one fetus whom we had not been able to diagnose as having trisomy 21

prenatally. Abnormal NT and morphological abnormalities could not be detected by our ultrasound examination during the whole pregnant period, and an increased DVPI during the first trimester was the only abnormal sign. It has already been reported that absent or reverse DV flow during atrial contraction was a significant predictor of aneuploidy, even in fetuses with normal NT.[6,22] However, it is controversial as to whether isolated abnormal DV flow should be an indication for invasive testing of fetal karyotypes.

These results suggest that abnormal DV flow should be a useful predictor of delivery, not only of infants with aneuploidy but also of SGA and LBW infants. Further studies are needed for determining which method is better, quantitative analysis such as the DVPI or a qualitative one (absent or reverse flow of DV).

The present study has two limitations. The first limitation is the accuracy of gestational age. The gestational age was adjusted by CRL measurement when estimated to be more than 7 days earlier than the date of the last menstrual period. Even in a normal fetus, the CRL has a variation of several days. In a fetus whose growth is already restricted early in the pregnancy, the gestational age may be shifted towards earlier than the actual age. The scientific validity of our study would have improved if the study population was restricted to women who had a regular 28-day menstrual cycle or to those who conceived through assisted reproductive technology. The second limitation was that we determined the normal references of UAPI and DVPI by lumping the measurement values to complete weeks disregarding days. These limitations may have affected our study but probably to a negligible degree because variation with gestational age is small (Table 2).

In conclusion, increased pulsation in the ductus venosus blood flow velocity is associated with increased risk of aneuploidy, but also fetal growth restriction.

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ORIGINAL ARTICLE

Establishment of reference ranges for ductus venosus waveform indices in the Japanese population

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Abstract
Objective To establish reference ranges for ductus venosus waveform indices in the Japanese population.
Methods In this retrospective cross-sectional study, 791 singleton fetuses of healthy Japanese couples were examined from January 2004 to January 2008. Reference ranges for ductus venosus waveform indices were constructed from cross-sectional data obtained at between 18 and 41 weeks of gestation.
Results With a success rate of 84%, a total of 667 measurements in 791 women were eligible for analysis. The median pulsatility index (PI) of fetal ductus venosus decreased from 0.54 at 18 weeks of gestation to 0.30 at 41 weeks of gestation. The median end-diastolic velocity/peak systolic velocity (a/S) of the ductus venosus increased from 0.56 at 18 weeks of gestation to 0.76 at 41 weeks of gestation.
Conclusions In this study, we established reference ranges for the PI and a/S of the ductus venosus in the Japanese population, which differed slightly from other published reference data. The results will be useful for further studies to determine the validity of the clinical importance of the ductus venosus for at-risk fetuses.

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Keywords Doppler velocimetry · Ductus venosus · Fetus · Reference range · Ultrasound

Introduction

Of all the precordial veins, the ductus venosus is the most commonly studied vessel. It yields the best and most reliable information on myocardial hemodynamics and functions of the fetal heart, with good reproducibility [1].

The ductus venosus is a precordial vein in the fetus that reflects the physiological status of the right ventricle. It appears to be an important regulator in the distribution of oxygen-rich blood between the liver and the heart. In the case of hypoxia, the fraction of ductus venosus shunting from the umbilical vein increases in order to maintain the oxygen supply to the brain and myocardium. Thus, the ductus venosus is important as a distributor of well-oxygenated blood to the fetal brain and myocardium [2–4].

Kiserud et al. [5] first reported Doppler ultrasound investigation of the ductus venosus as a diagnostic tool. Recent reports have indicated a relationship between the degree of acidemia and values of waveform indices of the ductus venosus due to chronic placental insufficiency associated with severe intrauterine growth restriction (IUGR), and have indicated a role in the diagnosis of fetal congestive cardiocirculatory diseases [6–8]. A relationship between perinatal outcome in early-onset IUGR and abnormalities of velocimetry in the ductus venosus has also been reported. In particular, the current standard management of IUGR involves Doppler examination of the ductus venosus [9–13].

Although many reference ranges have been established for the ductus venosus [1, 8, 14–21], most of the data reported were derived from Caucasian population-based