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Genetic association of aromatic hydrocarbon receptor (*AHR*) and cytochrome P450, family 1, subfamily A, polypeptide 1 (*CYP1A1*) polymorphisms with dioxin blood concentrations among pregnant Japanese women

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HIGHLIGHTS

- We examined the association of dioxin concentrations with genetic susceptibility.
- Six polymorphisms in genes encoding dioxin-metabolizing enzymes were investigated.
- These six polymorphisms were analyzed in 421 healthy pregnant Japanese women.
- We observed different blood concentrations and TEQs with both *AHR* (rs2066853) and *CYP1A1* (rs4646903).

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ABSTRACT

Dioxins are metabolized by cytochrome P450, family 1 (CYP1) via the aromatic hydrocarbon receptor (AHR). We determined whether different blood dioxin concentrations are associated with polymorphisms in *AHR* (dbSNP ID: rs2066853), AHR repressor (*AHRR*; rs2292596), CYP1 subfamily A polypeptide 1 (*CYP1A1*; rs4646903 and rs1048963), CYP1 subfamily A polypeptide 2 (*CYP1A2*; rs762551), and CYP1 subfamily B polypeptide 1 (*CYP1B1*; rs1056836) in pregnant Japanese women. These six polymorphisms were detected in 421 healthy pregnant Japanese women. Differences in dioxin exposure concentrations in maternal blood among the genotypes were investigated. Comparisons among the GG, GA, and AA genotypes of *AHR* showed a significant difference (genotype model: $P=0.016$ for the mono-*ortho* polychlorinated biphenyl concentrations and toxicity equivalence quantities [TEQs]). Second, we found a significant association with the dominant genotype model ([TT+TC] vs. CC: $P=0.048$ for the polychlorinated dibenzo-*p*-dioxin TEQs; $P=0.035$ for polychlorinated dibenzofuran TEQs) of *CYP1A1* (rs4646903). No significant differences were found among blood dioxin concentrations and polymorphisms in *AHRR*, *CYP1A1* (rs1048963), *CYP1A2*, and *CYP1B1*. Thus, polymorphisms in *AHR* and *CYP1A1* (rs4646903) were associated with maternal dioxin concentrations. However, differences in blood dioxin concentrations were relatively low.

Abbreviations: PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; PCB, polychlorinated biphenyl; TEQ, toxicity equivalence quantity; AHR, aromatic hydrocarbon receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; CYP1A1, cytochrome P450, family 1, subfamily A, polypeptide 1; CYP1A2, cytochrome P450, family 1, subfamily A, polypeptide 2; CYP1B1, cytochrome P450, family 1, subfamily B, polypeptide 1; AHRR, aromatic hydrocarbon receptor repressor; CYP, cytochrome P450; GSTT1, glutathione *S*-transferase θ1; GSTM1, glutathione *S*-transferase μ1; HexCB, Hexachlorinated biphenyl; PenCB, Pentachlorinated biphenyl; TEF, toxicity equivalence factor; SNPs, single-nucleotide polymorphisms; PenCB, pentachlorinated biphenyl; E_2, 17β-estradiol; E_1, estrone; 2-OH-E_2, 2-hydroxyestradiol; 4-OH-E_2, 4-hydroxyestradiol; ERα, estrogen receptor α; TSH, thyroid-stimulating hormone; TSHβ, thyroid-stimulating hormone, β subunit; E_2-ERα, 17β-estradiol-bound estrogen receptor α; T_3, triiodothyronine.

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1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs), which are all referred to as dioxins, are persistent endocrine-disrupting chemicals that bioaccumulate as a result of environmental exposure or ingestion of dioxin-containing foods. Adverse health effects of dioxin exposure in humans include the development of serious diseases such as diabetes and cancer and deleterious effects such as an altered immunological response and changes in the expression of receptors and metabolic enzymes (White and Birnbarm, 2009).

Low levels of dioxin exposure in pregnant women can have a significant effect on the developing fetus through circulating blood via the placenta (Miller et al., 2004; Chao et al., 2007). Exposure to high levels of PCDDs plus PCDFs (resulting in a median blood concentration of 168 pg/g lipid) in pregnant women is associated with decreased fundal length and uterine size in 8-year-old girls (Su et al., 2012). Exposure to high levels of PCDDs, PCDFs, and dioxin-like PCBs from dioxin-contaminated rice oil [mean blood concentration of 68.92 toxicity equivalence quantity (TEQ) pg/g lipid], which occurred in the late 1960s (Yusho disease), is associated with lower birth weight (Tsukimori et al., 2012). Additional studies have shown that exposure to low dioxin levels is associated with low birth weight (Tajimi et al., 2005; Sonneborn et al., 2008). One of our previous studies also showed that low prenatal dioxin exposure has a significant negative association with birth weight (Konishi et al., 2009). However, other studies have shown that pregnant women who are exposed to low dioxin levels do not give birth to babies with low birth weight (Longnecker et al., 2005; Nishijo et al., 2008). These conflicting results suggest that maternal genetic susceptibility regarding enzymes involved in dioxin metabolism may play a role.

Dioxins, which include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), bind the aromatic hydrocarbon receptor (AHR); are metabolized by cytochrome P450 (CYP)1, subfamily A, polypeptide 1 (CYP1A1), polypeptide 2 (CYP1A2), and subfamily B, polypeptide 1 (CYP1B1); and stimulate the transcription suppressor factor AHR repressor (AHRR). Genetic polymorphisms in *AHR*, *AHRR*, and *CYP* modulate the degree of disease risk. For example, a polymorphism in *AHR* (G > A, Arg554Lys, dbSNP ID: rs2066853) is associated with survival in soft-tissue sarcoma (Berwick et al., 2004). A polymorphism in *AHRR* (C > G, Pro185Ala, rs2292596) is associated with endometriosis (Tsuchiya et al., 2005; Kim et al., 2007). A polymorphism in *CYP1A1* (T > C, *Msp*I, rs4646903) is associated with polycystic ovary syndrome (Babu et al., 2004) and lung cancer (Song et al., 2001). A polymorphism in *CYP1A1* (A > G, Ile462Val, rs1048963) is associated with lung cancer (Sugimura et al., 1995). A polymorphism in *CYP1A2* (A > C, *CYP1A2*1F*, rs762551) is associated with squamous cell carcinoma (Singh et al., 2010) and breast cancer (Shimada et al., 2009). Finally, a polymorphism in *CYP1B1* (C > G, Leu432Val, rs1056836) is associated with breast cancer (Shimada et al., 2009). Disease and the effect of exposure concentration are not independent phenomena. First, various polymorphisms may affect dioxin blood concentrations. Second, the exposure concentration may affect the reproductive and immune systems. Third, effects on these systems may lead to increased risk for various diseases.

Exposure to low levels of dioxins may cause reproductive toxicity (Tajimi et al., 2005; Sonneborn et al., 2008; Konishi et al., 2011). Through AHR and the CYP1 family of enzymes, dioxins share a metabolic pathway with polycyclic aromatic hydrocarbons, which are components of cigarette smoke. The risk of fetal growth restriction in pregnant women who smoke during pregnancy is modulated by maternal polymorphisms in *CYP1A1*, glutathione *S*-transferase θ1 (*GSTT1*), and glutathione *S*-transferase μ1 (*GSTM1*) (Delpisheh et al., 2009). Similarly, differences in genetic susceptibility to environmental chemicals in the parental generation may cause adverse health effects in the offspring. Maternal genotypes consisting of *GSTM1* null, a *CYP1A1* (rs1048963) variant, and the combination of *GSTM1* null and a *CYP1A1* (rs4646903) variant are associated with increased risk for low birth weight and premature birth (Sram et al., 2006). Genotypes can modify the effects of environmental factors. Therefore, the genetic susceptibility of pregnant women to environmental chemicals may affect the health status of the next generation.

Our understanding of the association between environmental exposure to chemicals, including dioxins, and its effect on fetal and childhood development years after birth is, however, limited. Dioxin-like PCB (IUPAC No. 126) is ~10,000-fold more potent than non-dioxin-like PCB (IUPAC No.153) in pregnancy. Isomers of these compounds impair learning in young (3-month-old) rats, and the effects are similar in both males and females (Piedrafita et al., 2008). However, the underlying mechanisms in humans remain unclear. In the future, we will investigate the effects of dioxins on developing school-aged children. We also need to examine the associations between dioxin concentrations and polymorphisms in dioxin-metabolizing genes and evaluate the gene–environment interactions. Consequently, here we examined the association of dioxin concentrations in the blood with genetic susceptibility in healthy mothers. The objective of this study was to look for differences in exposure concentrations of dioxins and *AHR* (rs2066853), *AHRR* (rs2292596), *CYP1A1* (rs4646903 and rs1048963), *CYP1A2* (rs762551), and *CYP1B1* (rs1056836) genotypes.

2. Materials and methods

2.1. Study population

From July 2002 through July 2004, we enrolled pregnant women from Sapporo Toho Hospital in Hokkaido, northern Japan, after obtaining their informed consent. Details of the cohort study methods have been reported (Kishi et al., 2011). A total of 514 mothers were registered, but 10 were excluded because of miscarriage, stillbirth, relocation, or voluntary withdrawal from the study before follow-up. Participants completed a self-administered questionnaire after the second trimester of pregnancy regarding dietary habits, alcohol intake, smoking status, caffeine intake, household income, educational level, and medical history. Information from maternal medical records concerning pregnancy complications and parity was obtained. In the present study, 422 complete sets of dioxin congener concentrations and polymorphisms were selected from the 514 registered participants of the cohort study and were used for chemical analysis. However, one sample was excluded from the study because the PCDF concentrations were extremely high and the Smirnoff-Grubbs rejection test was significant. The Institutional Ethical Board for Human Gene and Genome Studies of Hokkaido University Graduate School of Medicine approved the study protocol.

2.2. Sample collection and dioxin analysis

Sample collection has been described in detail elsewhere (Kishi et al., 2011). Analyses of dioxins were performed as described (Todaka et al., 2003). Briefly, a 40-ml blood sample was taken from the maternal peripheral vein during the third trimester. If blood could not be drawn during pregnancy because of anemia, we obtained the blood during hospitalization within a week after delivery. All samples were stored at −80 °C until analysis. PCDD, PCDF, and dioxin-like PCB concentrations in the blood were measured using high-resolution gas chromatography/high-resolution mass spectrometry at the Fukuoka Institute of Health and Environmental Sciences. Sample values below the detection limit were assigned a value of one-half the detection limit to estimate the total dioxin concentration. TEQ values were calculated by multiplying the concentrations of each congener by its toxicity equivalence factor (TEF) value based on the 2006 World Health Organization standards (Van den Berg et al., 2006). We measured the dioxin concentrations in 426 maternal blood samples.

2.3. Genetic analysis

We evaluated six single-nucleotide polymorphisms (SNPs), namely *AHR* (G > A, rs2066853), *AHRR* (C > G, rs2292596), *CYP1A1* (T > C, rs4646903; A > G, rs1048963), *CYP1A2* (A > C, rs762551), and *CYP1B1* (C > G, rs1056836). Genomic DNA was extracted from 400 μl of maternal blood using a Maxwell 16 Instrument (Promega Corporation, Madison, WI, USA). DNA amplifications were performed in batches

in a 96-well microamp reaction plate using validated TaqMan probes for each of the six SNPs on a Gene Amp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) with an end-point allelic discrimination assay (Ranade et al., 2001) on a 7300/7500 Real-time PCR System (Applied Biosystems). We randomly selected 20 samples and repeated genotyping to check for genotyping quality. The results were 100% concordant.

2.4. Statistical analysis

Descriptive statistics for pregnant women are expressed as the mean ± standard deviation, as the median (range), or as numbers (percentages). The dioxin and dioxin-like PCB concentrations were lipid adjusted (pg/g lipid) and assumed to have a value equal to half the limit of detection when the levels were below the limit of detection for individual congeners. Associations among dioxin concentrations, TEQ, and genotypes of *AHR* (rs2066853), *AHRR* (rs2292596), *CYP1A1* (rs4646903 and rs1048963), *CYP1A2* (rs762551), and *CYP1B1* (rs1056836) were analyzed with a generalized linear model adjusted for maternal age, maternal height, maternal weight before pregnancy, caffeine intake during pregnancy, alcohol consumption during pregnancy, parity, maternal smoking status during pregnancy, maternal educational level, annual household income, inshore fish intake during pregnancy, deep-sea fish intake during pregnancy, and blood sampling period. *P*-values were calculated for a genotype model, a dominant model, and a recessive model. The dominant model consisted of the following: (AA+AG) vs. GG for *AHR*; (CC+CG) vs. GG for *AHRR*; (TT+TC) vs. CC for *CYP1A1* (rs4646903); (AA+AG) vs. GG for *CYP1A1* (rs1048963); (CC+AC) vs. AA for *CYP1A2*; and (GG+GC) vs. CC for *CYP1B1*. The recessive model was as follows: AA vs. (AG+GG) for *AHR*; CC vs. (CG+GG) for *AHRR*; TT vs. (TC+CC) for *CYP1A1* (rs4646903); AA vs. (AG+GG) for *CYP1A1* (rs1048963); CC vs. (AA+AC) for *CYP1A2*; and GG vs. (GC+CC) for *CYP1B1* (Klein et al., 2010; Qiu et al., 2010; Yu et al., 2012; Xie et al., 2012; Luo et al., 2013).

All statistical analyses were performed using SPSS 15.0 statistical software (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered significant.

3. Results

Demographic characteristics of the participants are shown in Table 1. The mean age, height, and weight before pregnancy were 30.8 years, 158.2 cm, and 53.2 kg, respectively. The percentages of participants who drank alcohol and smoked during pregnancy were 30.4% and 17.1%, respectively. The majority of participants had 13–16 years of education (55.8%), 3–5 million yen as their annual household income (49.6%), consumed inshore fish 1–2 times/month (49.9%) and deep-sea fish 1–2 times/week (47.7%), and had their blood taken during pregnancy (69.6%).

The distributions of the *AHR* (rs2066853), *AHRR* (rs2292596), *CYP1A1* (rs4646903 and rs1048963), *CYP1A2* (rs762551), and *CYP1B1* (rs1056836) polymorphisms are shown in Table 2. No significant deviation of genotype frequencies from the Hardy-Weinberg equilibrium was detected in the SNPs (data not shown). The *AHR* (G>A), *AHRR* (C>G), *CYP1A1* (T>C, rs4646903; A>G, rs1048963), *CYP1A2* (A>C), and *CYP1B1* (C>G) polymorphisms showed minor allele frequencies of 43.1%, 39.8%, 34.3%, 22.1%, 37.2%, and 13.4%, respectively, among the pregnant Japanese women in this study.

Tables 3 and 4 show the adjusted mean concentrations (with 95% confidence intervals) and TEQs in the generalized linear model for total PCDDs, PCDFs, and dioxin-like PCBs among *AHR* (rs2066853) (Table 3) and *CYP1A1* (rs4646903) (Table 4) polymorphisms for pregnant women in Sapporo, Hokkaido, Japan. Figs. 1 and 2 show the adjusted mean concentrations (Fig. 1) and TEQs (Fig. 2) in the generalized linear model of congeners.

Comparison among GG, GA, and AA of *AHR* (rs2066853) showed a significant difference (genotype model: $P = 0.016$ for the mono-*ortho* PCB concentrations and TEQ; $P = 0.014$ for the total dioxin concentrations). In addition, we also found a significant association in the dominant genotype model GG vs. (GA+AA): $P = 0.047$ for PCDD concentrations; $P = 0.028$ for non-*ortho* PCB concentrations; $P = 0.022$ for non-*ortho* PCB TEQ; $P = 0.004$ for mono-*ortho* PCB concentrations, TEQ, and total dioxin concentrations (Table 3).

A comparison among TT, TC, and CC of *CYP1A1* (rs4646903) showed no significant difference. However, we did find a significant association in the dominant genotype model (TT+TC) vs. CC: $P = 0.048$ for PCDD TEQ; $P = 0.035$ for PCDF TEQ (Table 4).

Table 1
Characteristics of the study population in Sapporo, Hokkaido, Japan.

Characteristic	Value ($n = 421$)[a]
Maternal age (years)	30.8 ± 4.7
Maternal height (cm)	158.2 ± 5.4
Maternal weight before pregnancy (kg)	53.2 ± 8.8
Caffeine intake during pregnancy (mg/day)	117.3 (1.5–646.3)
Alcohol intake during pregnancy	
Yes	128 (30.4%)
No	293 (69.6%)
Alcohol consumption of the drinkers (g/day)	1.2 (0.3–51.8)
Parity	
Primiparous	204 (48.5%)
Multiparous	217 (51.5%)
Maternal smoking status during pregnancy	
Yes	72 (17.1%)
No	349 (82.9%)
Education level (years)	
≤9	9 (2.1%)
10–12	168 (39.9%)
13–16	235 (55.8%)
≥17	9 (2.1%)
Annual household income (million yen)	
≤3	68 (16.2%)
4–5	209 (49.6%)
6–7	93 (22.1%)
8–10	44 (10.5%)
>10	7 (1.7%)
Inshore fish intake during pregnancy	
Never	20 (4.8%)
1–2 times/month	210 (49.9%)
1–2 times/week	167 (39.7%)
3–4 times/week	23 (5.5%)
Almost every day	1 (0.2%)
Deep-sea fish intake during pregnancy	
Never	12 (2.9%)
1–2 times/month	182 (43.2%)
1–2 times/week	201 (47.7%)
3–4 times/week	25 (5.9%)
Almost every day	1 (0.2%)
Blood sampling period	
During pregnancy	293 (69.6%)
Postpartum	128 (30.4%)

[a] Data are presented as *n* (%), mean ± standard deviation, or median (range).

In a stratified analysis by congener, concentrations of the dioxins 2,3′,4,4′,5-pentachlorinated biphenyl (PenCB; IUPAC No. 118), 2,3,3′,4,4′-PenCB (IUPAC No. 105), and 2,3′,4,4′,5,5′-hexachlorinated biphenyl (HexCB; IUPAC No. 167) of the *AHR* (G>C, Arg554Lys) genotype model and dominant model showed a significant difference (genotype model [GG vs. GA] and dominant model GG vs. [GA+AA]): $P = 0.008$ and $P = 0.002$ for 2,3′,4,4′,5-PenCB (IUPAC No. 118) concentration; $P = 0.009$ and $P = 0.002$ for 2,3,3′,4,4′-PenCB (IUPAC No. 105) concentration; and $P = 0.035$ and $P = 0.011$ for 2,3′,4,4′,5,5′-HexCB (IUPAC No. 167) concentrations, respectively. Furthermore, 2,3,4,7,8-Pentachlorinated dibenzofuran (PeCDF) concentrations in the *CYP1A1* (T>C, *MspI*) genotype model and dominant model were significantly different (genotype model TT vs. CC and dominant model [TT+TC] vs. CC): $P = 0.049$ and $P = 0.028$, respectively (Fig. 1). In a stratified analysis by congener, TEQs of the dioxins, 2,3′,4,4′,5-PenCB (IUPAC No. 118), 2,3,3′,4,4′-PenCB (IUPAC No. 105), and 2,3′,4,4′,5,5′-HexCB (IUPAC No. 167) of the *AHR* (G>C, Arg554Lys) genotype model and dominant model were significantly different (genotype model GG vs. GA and dominant model GG vs. [GA+AA]): $P = 0.008$ and $P = 0.002$

Table 2
Genotype frequency of *AHR*, *AHRR*, *CYP1A1*, *CYP1A2*, and *CYP1B1* polymorphisms among pregnant women in Sapporo, Hokkaido, Japan.

Genotype	Pregnant women (n = 421) (%)
***AHR* (G > A, Arg554Lys, dbSNP ID: rs2066853)**	
GG	142 (33.7)
GA	195 (46.3)
AA	84 (20.0)
GG + GA	337 (80.0)
GA + AA	279 (66.3)
G allele	479 (56.9)
A allele	363 (43.1)
***AHRR* (C > G, Pro185Ala, dbSNP ID: rs2292596)**	
CC	145 (34.4)
CG	217 (51.5)
GG	59 (14.0)
CC + CG	362 (86.0)
CG + GG	276 (65.6)
C allele	507 (60.2)
G allele	335 (39.8)
***CYP1A1* (T > C, *MspI*, dbSNP ID: rs4646903)**	
TT	176 (41.8)
TC	201 (47.7)
CC	44 (10.5)
TT + TC	377 (89.5)
TC + CC	245 (58.2)
T allele	553 (65.7)
C allele	289 (34.3)
***CYP1A1* (A > G, Ile462Val, dbSNP ID: rs1048963)**	
AA	253 (60.1)
AG	150 (35.6)
GG	18 (4.3)
AA + AG	403 (95.7)
AG + GG	168 (39.9)
A allele	656 (77.9)
G allele	186 (22.1)
***CYP1A2* (A > C, *CYP1A2*1F*, dbSNP ID: rs762551)**	
AA	169 (40.1)
AC	191 (45.4)
CC	61 (14.5)
AA + AC	360 (85.5)
AC + CC	252 (59.9)
A allele	529 (62.8)
C allele	313 (37.2)
***CYP1B1* (C > G, Leu432Val, dbSNP ID: rs1056836)**	
CC	317 (75.3)
CG	95 (22.6)
GG	9 (2.1)
CC + CG	412 (97.9)
CG + GG	104 (24.7)
C allele	729 (86.6)
G allele	113 (13.4)

for 2,3′,4,4′,5-PenCB (IUPAC No. 118) concentration, $P = 0.014$ and $P = 0.002$ for 2,3,3′,4,4′-PenCB (IUPAC No. 105) concentration, and $P = 0.043$ and $P = 0.013$ for 2,3′,4,4′,5,5′-HexCB (IUPAC No. 167) concentration, respectively. Furthermore, 2,3,4,7,8-PeCDF TEQs of the *CYP1A1* (T > C, *MspI*) genotype model and dominant model were significantly different (genotype model TT vs. CC and dominant model [TT + TC] vs. CC): $P = 0.045$ and $P = 0.028$, respectively (Fig. 2).

In contrast, no significant differences were obtained for dioxin concentrations or TEQs among the *AHRR* (rs2292596), *CYP1A1* (rs1048963), *CYP1A2* (rs762551), and *CYP1B1* (rs1056836) polymorphisms (data not shown).

4. Discussion

Recent investigations from the "Hokkaido Study on Environment and Children's Health" have indicated that prenatal exposure to dioxins affects birth weight (Konishi et al., 2009), mental and motor development at the age of 6 months (Nakajima et al., 2006), and otitis media at the age of 18 months (Miyashita et al., 2011). Furthermore, maternal smoking and metabolism-related genes such as *AHR*, *CYP1A1*, *GSTM1*, NADPH dehydrogenase, quinine 1 (*NQO1*), methylenetetrahydrofolate reductase (*MTHFR*), and CYP2 subfamily E polypeptide 1 (*CYP2E1*) affect infant birth size (Sasaki et al., 2006, 2008; Yila et al., 2012).

TCDD is the most toxic of all dioxin compounds. TCDD is used as a standard to evaluate the TEF value of dioxins and dioxin-like congeners to indicate the degree of toxicity. This TEF is determined by the sensitivity of AHR (Van den Berg et al., 1998). Dioxins including TCDD are sensitive to AHR. Although the toxic effects of TCDD have been studied for several decades, the detailed molecular mechanisms are still poorly understood except for the TCDD-mediated transcriptional regulation of AHR and its binding with AHR nuclear translocator (Gim et al., 2010). TCDD accumulates in fatty tissue, stimulates AHR activation, and causes transcription of *CYP1A1*, *CYP1A2*, *CYP1B1*, and *AHRR* (Mimura and Fujii-Kuriyama, 2003). *CYP1A1* is the most potently induced gene following AHR activation (Barouki et al., 2007). CYP1A1 is associated with metabolic activation of hydrophobic molecules such as PCDDs (Ziegler, 1991). The catalytic activities of CYP1B1 overlap with those of CYP1A1 and CYP1A2 (Shimada et al., 1997).

TCDD modulates the induction of DNA strand breaks and poly(adenosine diphosphate ribose) polymerase-1 activation by 17β-estradiol in human breast carcinoma cells by altering CYP1A1 and CYP1B1 expression (Lin et al., 2008). CYP1A1 and CYP1B1 mediate the transformation of 17β-estradiol (E_2)/estrone (E_1) to the biologically active metabolites 2-hydroxyestradiol (2-OH-E_2) and 4-hydroxyestradiol (4-OH-E_2) (Hayes et al., 1996; Martucci and Fishman, 1993; Spink et al., 1997). TCDD enhances the biotransformation of E_2 to 2-OH-E_2 and 4-OH-E_2 in human MCF-7 breast cancer cells (Lavigne et al., 2001). Both 2-OH-E_2 and 4-OH-E_2 induce oxidative damage in purified DNA and break DNA into single strands (Miura et al., 2000; Lin et al., 2003). Cells treated with E_2 and 2-OH-E_2 exhibit a significant decrease in the estrogen-induced response (Gupta et al., 1998).

TCDD mediates estrogen receptor α (ERα) signaling in MCF-7 cells under moderately hypoxic conditions (Seifert et al., 2009). In the mouse uterus and in breast cancer cells, ERα levels are significantly lower after treatment with estradiol plus TCDD than with TCDD alone, indicating that AHR-mediated inhibition occurs by estradiol-induced transactivation. TCDD induces an interaction between AHR and ERα in the presence of estradiol (Wormke et al., 2003).

E_2-ERα inhibits thyroid-stimulating hormone, β subunit (*TSHβ*) expression (Nagayama et al., 2008). Transcriptional repression of *TSHβ* is specific to triiodothyronine (T_3) and its receptor. The proinflammatory cytokine interleukin-1β decreases transcription of the thyroid hormone receptor α gene in liver cells (Kwakkel et al., 2007).

An adequate supply of cerebral T_3 is needed by the fetus. Thyroid hormone-dependent neurodevelopment begins in the second half of the first trimester of pregnancy. The reserves of the fetal gland are low during this period, and thus most of the thyroid hormones needed by the fetus before birth are contributed by the mother (Skeaff, 2011). Effects that are due to a lack of thyroid hormones in pregnant women with poor dioxin-metabolizing enzyme activity may impair fetal brain development and contribute to hypothyroidism in the fetus.

To the best of our knowledge, this is the first study to show different dioxin blood levels in women with both *AHR* (rs2066853) and *CYP1A1* (rs4646903) polymorphisms. Activation mediated by AHR and CYP1A1 is an important mechanism for metabolizing dioxins. The homozygous *AHR* (rs2066853) variant genotype (AA) is associated with significantly lower mRNA expression of *AHR*, *ARNT*, and *CYP1B1* (Helmig et al., 2011). *AHR* AA may thus reduce AHR activity

Table 3
Adjusted means in the generalized linear model of total PCDDs, PCDFs, and dioxin-like PCBs among *AHR* polymorphisms (G > A, Arg554Lys, dbSNP ID: rs2066853) of pregnant women in Sapporo, Hokkaido, Japan.

Model[a]	GG[b]	GA[b]	AA[b]	GA + AA[b]	GG + GA[b]	*P*-value
Concentration (pg/g lipid)						
PCDDs						
Genotype[a]	478.5 (444.1–512.9)	519.7 (490.5–548.9)	526.3 (481.6–570.9)			0.097
Dominant[a]	478.5 (444.1–512.9)			521.7 (497.3–546.0)		0.047*
Recessive[a]			526.3 (481.6–570.9)		502.4 (480.3–524.5)	0.355
PCDFs						
Genotype[a]	19.2 (17.3–21.1)	21.0 (19.4–22.7)	20.2 (17.7–22.7)			0.365
Dominant[a]	19.2 (17.3–21.1)			20.8 (19.4–22.1)		0.189
Recessive[a]			20.2 (17.7–22.7)		20.3 (19.0–21.5)	0.968
Non-*ortho* PCBs						
Genotype[a]	74.6 (67.7–81.5)	83.4 (77.6–89.3)	86.1 (77.2–95.1)			0.079
Dominant[a]	74.6 (67.7–81.5)			84.2 (79.4–89.1)		0.028*
Recessive[a]			86.1 (77.2–95.1)		79.7 (75.3–84.2)	0.216
Mono-*ortho* PCBs						
Genotype[a]	11,266.3 (10,265.9–12,266.8)	13,146.5 (12,297.1–13,995.9)	12,948.9 (11,650.1–14,247.7)			0.016*
Dominant[a]	11,266.3 (10,265.9–12,266.8)			13,087.0 (12,379.6–13,794.4)		0.004*
Recessive[a]			12,948.9 (11,650.1–14,247.7)		12,356.1 (11,709.3–13,003.3)	0.434
Total dioxins						
Genotype[a]	11,838.7 (10,820.5–12,856.9)	13,770.7 (12,906.1–14,635.2)	13581.5 (12,259.1–14,904.3)			0.014*
Dominant[a]	11,838.7 (10,820.5–12,856.9)			13,713.7 (12,993.7–14,433.7)		0.004*
Recessive[a]			13581.5 (12,259.1–14,904.3)		12,958.9 (12,300.1–13,617.0)	0.419
TEQ (pg/g lipid)						
PCDDs						
Genotype[a]	7.003 (6.513–7.493)	7.465 (7.050–7.881)	7.472 (6.837–8.108)			0.323
Dominant[a]	7.003 (6.513–7.493)			7.467 (7.121–7.814)		0.132
Recessive[a]			7.472 (6.837–8.108)		7.271 (6.957–7.585)	0.583
PCDFs						
Genotype[a]	2.505 (2.342–2.668)	2.598 (2.460–2.736)	2.571 (2.359–2.782)			0.696
Dominant[a]	2.505 (2.342–2.668)			2.590 (2.475–2.705)		0.410
Recessive[a]			2.571 (2.359–2.782)		2.559 (2.455–2.664)	0.927
Non-*ortho* PCBs						
Genotype[a]	4.179 (3.769–4.590)	4.809 (4.460–5.157)	4.693 (4.160–5.226)			0.068
Dominant[a]	4.179 (3.769–4.590)			4.774 (4.484–5.064)		0.022*
Recessive[a]			4.693 (4.160–5.226)		4.544 (4.280–4.809)	0.633
Mono-*ortho* PCBs						
Genotype[a]	0.338 (0.308–0.368)	0.394 (0.369–0.420)	0.388 (0.350–0.427)			0.016*
Dominant[a]	0.338 (0.308–0.368)			0.393 (0.371–0.414)		0.004*
Recessive[a]			0.388 (0.350–0.427)		0.371 (0.351–0.390)	0.434
Total dioxins						
Genotype[a]	14.025 (13.056–14.995)	15.267 (14.443–16.090)	15.124 (13.865–16.383)			0.145
Dominant[a]	14.025 (13.056–14.995)			15.224 (14.538–15.910)		0.050
Recessive[a]			15.124 (13.865–16.383)		14.745 (14.121–15.369)	0.604

The generalized linear model was adjusted for maternal age, maternal height, maternal weight before pregnancy, caffeine intake during pregnancy, alcohol consumption during pregnancy, parity, maternal smoking status during pregnancy, maternal educational level, annual household income, inshore fish intake during pregnancy, deep-sea fish intake during pregnancy, and blood sampling period.

[a] Model types are as follows: Genotype, genotype model; Dominant, dominant genotype model; Recessive, recessive genotype model.

[b] 95% CI, 95% confidence interval.

* Statistically significant values ($P < 0.05$).

and decrease metabolism by CYP1. CYP1A1 activity is significantly higher in people with the CYP1A1 (rs4646903) TC or CC genotype (Landi et al., 1994). Dioxin levels may be influenced by CYP1A1 activity or CYP1A1 expression.

In our previous studies, we noted a decrease in birth weight of 231.5 g and 258.8 g with a 10-fold increase in the TEQ levels of total PCDDs and PCDFs, respectively (Konishi et al., 2009). In addition, total PCDD concentrations were significantly negatively associated with Bayley scales of infant development-II mental development index scores at 6 months of age [$\beta = -0.234$ was the point increase in development score per total PCDD level (natural logarithm)] (Nakajima et al., 2006). The odds ratio was 2.50 for otitis media for the 75–100th percentiles of TEQ (3.06–7.77 TEQ pg/g lipid) of total PCDFs increases as compared with the 0–25th percentiles of TEQ (0.64–1.79 TEQ pg/g lipid) (Miyashita et al., 2011). With respect to different polymorphisms, decreases in birth weight and length of 211 g and 1.2 cm, respectively, were noted for infants born to women who smoked during pregnancy with *AHR* (G > A,

Table 4
Adjusted means in the generalized linear model of total PCDDs, PCDFs, and dioxin-like PCBs among *CYP1A1* polymorphisms (T > C, *MspI*, dbSNP ID: rs4646903) of pregnant women in Sapporo, Hokkaido, Japan.

Model[a]	TT[b]	TC[b]	CC[b]	TC + CC[b]	TT + TC[b]	*P*-value[c]
Concentration (pg/g lipid)						
PCDDs						
Genotype[a]	529.8 (499.3–560.3)	497.2 (468.6–525.7)	461.9 (400.6–523.1)			0.097
Dominant[a]			461.9 (400.6–523.1)		512.4 (491.6–533.2)	0.127
Recessive[a]	529.8 (499.3–560.3)			490.9 (465.0–516.7)		0.057
PCDFs						
Genotype[a]	20.8 (19.1–22.5)	20.4 (18.8–21.9)	17.9 (14.4–21.3)			0.324
Dominant[a]			17.9 (14.4–21.3)		20.5 (19.4–21.7)	0.144
Recessive[a]	20.8 (19.1–22.5)			19.9 (18.5–21.3)		0.454
Non-*ortho* PCBs						
Genotype[a]	85.5 (79.4–91.6)	78.6 (72.9–84.3)	74.0 (61.6–86.3)			0.139
Dominant[a]			74.0 (61.6–86.3)		81.8 (77.6–86.0)	0.240
Recessive[a]	85.5 (79.4–91.6)			77.8 (72.6–83.0)		0.061
Mono-*ortho* PCBs						
Genotype[a]	12,748.0 (11,851.9–13,644.0)	12,354.8 (11,517.0–13,192.7)	11,911.9 (10,112.4–13,711.4)			0.666
Dominant[a]			11,911.9 (10,112.4–13,711.4)		12,538.3 (11,928.2–13,148.3)	0.518
Recessive[a]	12,748.0 (11,851.9–13,644.0)			12,275.6 (11,517.7–13,033.5)		0.431
Total dioxins						
Genotype[a]	13,384.0 (12,472.0–14,296.1)	12,951.0 (12,098.2–13,803.8)	12,465.6 (10,634.0–14,297.2)			0.623
Dominant[a]			12,465.6 (10,634.0–14,297.2)		13,153.0 (12,532.0–13,774.1)	0.486
Recessive[a]	13,384.0 (12,472.0–14,296.1)			12,864.1 (12,092.7–13,635.6)		0.394
TEQ (pg/g lipid)						
PCDDs						
Genotype[a]	7.616 (7.183–8.049)	7.225 (6.821–7.630)	6.480 (5.611–7.349)			0.062
Dominant[a]			6.480 (5.611–7.349)		7.408 (7.113–7.703)	0.048*
Recessive[a]	7.616 (7.183–8.049)			7.092 (7.182–8.049)		0.072
PCDFs						
Genotype[a]	2.653 (2.510–2.797)	2.545 (2.411–2.680)	2.267 (1.978–2.555)			0.061
Dominant[a]			2.267 (1.978–2.555)		2.596 (2.498–2.694)	0.035*
Recessive[a]	2.653 (2.510–2.797)			2.495 (2.373–2.617)		0.103
Non-*ortho* PCBs						
Genotype[a]	4.730 (4.363–5.096)	4.496 (4.154–4.839)	4.300 (3.564–5.035)			0.490
Dominant[a]			4.300 (3.564–5.035)		4.605 (4.356–4.855)	0.441
Recessive[a]	4.730 (4.363–5.096)			4.461 (4.152–4.771)		0.273
Mono-*ortho* PCBs						
Genotype[a]	0.382 (0.356–0.409)	0.371 (0.346–0.396)	0.357 (0.303–0.411)			0.666
Dominant[a]			0.357 (0.303–0.411)		0.376 (0.358–0.394)	0.518
Recessive[a]	0.382 (0.356–0.409)			0.368 (0.346–0.391)		0.431
Total dioxins						
Genotype[a]	15.381 (14.521–16.242)	14.638 (13.833–15.442)	13.403 (11.676–15.131)			0.111
Dominant[a]			13.403 (11.676–15.131)		14.985 (14.398–15.571)	0.090
Recessive[a]	15.381 (14.521–16.242)			14.417 (13.688–15.146)		0.095

[a] Model types are as follows: Genotype, genotype model; Dominant, dominant genotype model; Recessive, recessive genotype model.
[b] 95% CI, 95% confidence interval.
* Statistically significant values ($P < 0.05$).

Arg554Lys) GG as compared with those born to women who did not smoke during pregnancy with *AHR* GA + AA. Decreases in birth weight and length of 170 g and 0.8 cm, respectively, were noted for infants born to women who smoked during pregnancy with *CYP1A1* (T > C, *MspI*) TC + CC as compared with those born to women who did not smoke during pregnancy with *CYP1A1* TT. Decreases in birth weight and length of 315 g and 1.7 cm, respectively, were noted for infants born to women who smoked during pregnancy with *AHR* GG, *CYP1A1* TC + CC as compared with those born to women who did not smoke during pregnancy with *AHR* GA + AA, *CYP1A1* TT (Sasaki et al., 2006).

In 82 children aged 6–10 years who were attending schools near an industrial area in Mexico, Sánchez-Guerra et al. (2012) investigated the association among *CYP1A1*2C*, *CYP1B1*3*, *GSTM1*0*, and *GSTT1*0* polymorphisms, urinary 1-hydroxypyrene (1-OHP; a biomarker of polycyclic aromatic hydrocarbon exposure), and DNA adducts. They observed higher urinary 1-OHP concentrations in those with *CYP1A1*2C* AG + GG as compared with those with *CYP1A1*2C* AA (0.23 μmol/mol creatinine for AA vs. 0.45 μmol/mol creatinine for AG + GG).

In human full-term placental trophoblast cultures, after archetype AHR ligands/activators (2,3,7,8-TCDD and

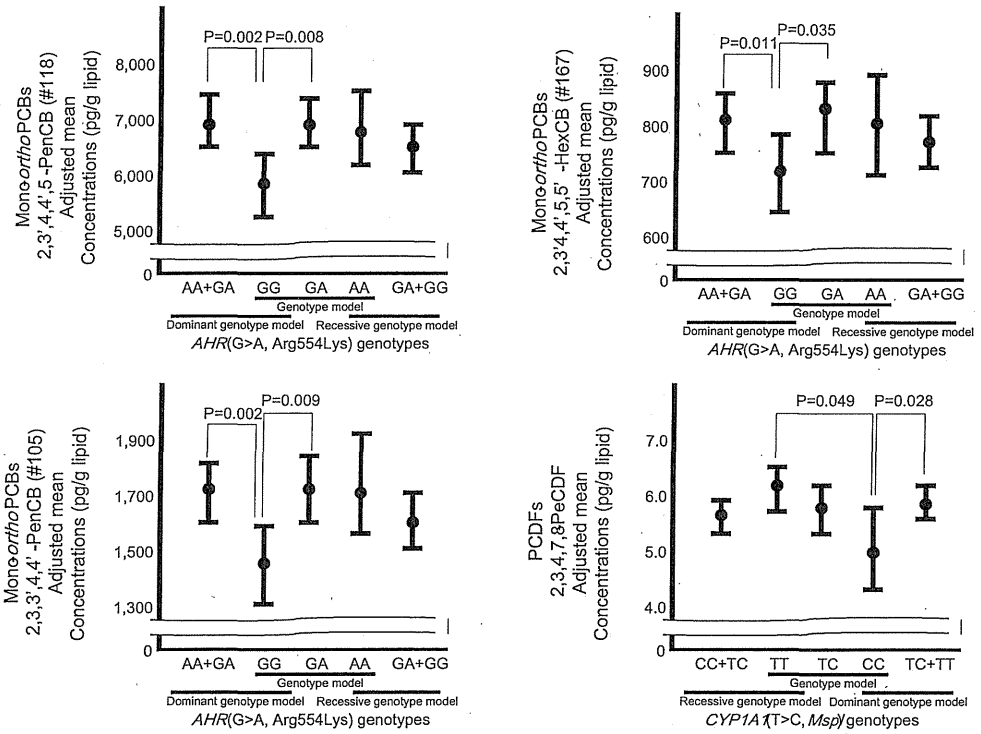

Fig. 1. Concentrations for 2,3′,4,4′,5-PenCB (IUPAC No. 118), 2,3,3′,4,4′,-PenCB (IUPAC No. 105), 2,3′,4,4′,5,5′-HexCB (IUPAC No. 167) and 2,3,4,7,8-PeCDF in the generalized linear model of dioxin congeners among *AHR* and *CYP1A1* polymorphisms of pregnant women in Sapporo, Hokkaido, Japan.
Dots and bars are the adjusted means and 95% confidence intervals, respectively. The means were adjusted for maternal age, maternal height, maternal weight before pregnancy, caffeine intake during pregnancy, alcohol consumption during pregnancy, parity, maternal smoking status during pregnancy, maternal educational level, annual household income, inshore fish intake during pregnancy, deep-sea fish intake during pregnancy, and blood sampling period in the generalized linear model.

3-methylcholanthlene) were added, CYP1A1 mRNA, but not CYP1A2, CYP1B1, AHR, or AHRR mRNA, was significantly induced (Stejskalova et al., 2011). In the present study, dioxin-like PCB concentrations and TEQ were associated with a significant reduction in the frequency of *AHR* (G>A, Arg554Lys) GA+AA as compared with GG. PCDFs were associated with a significant reduction in the frequency of CYP1A1 (T>C, *MspI*) TC+CC as compared with TT. After adjusting for smoking status during pregnancy, changes in dioxin concentrations and TEQ were significantly decreased in association with *AHR* and *CYP1A1* polymorphisms, but not with *AHRR*, *CYP1A2*, or *CYP1B1*. Compared with previous studies by Sasaki et al. (2006), Sánchez-Guerra et al. (2012), and Stejskalova et al. (2011), we observed statistically significant differences only in *AHR* and *CYP1A1*, and not in *AHRR*, *CYP1A2*, or *CYP1B1*, which is similar to the previous three reports. It may be that the chemical effects of tobacco smoke, which include polycyclic aromatic hydrocarbons, are more important confounding factors for *AHR* and *CYP1A1* genotypes in pregnant women who are exposed to low levels of dioxins. However, the importance of associations between the *AHR* (G>A, Arg554Lys) or *CYP1A1* (T>C, *MspI*) genotype and dioxin concentrations in humans remains unclear. In our study, we observed differences of ~1.1-fold in dioxin TEQs and concentrations according to genotypes. Based on our previous study (Konishi et al., 2009), changes in birth weight of about −20 to −25 g (maximum levels) will predict a 1.1-fold increase in the levels of dioxins. For pregnant Japanese women, TEQs of 2,3′,4,4′,5-PenCB (IUPAC No. 118), 2,3,3′,4,4′-PenCB (IUPAC No. 105), and 2,3′,4,4′,5,5′-HexCB (IUPAC No. 167) showed significant differences in the *AHR* genotypes. The metabolism and pharmacokinetics of 2,3′,4,4′,5-PenCB (IUPAC No. 118), 2,3,3′,4,4′-PenCB (IUPAC No. 105), and 2,3′,4,4′,5,5′-HexCB (IUPAC No. 167) are unclear in humans, but some mouse studies have been performed. Typically, one dose–response relationship was observed for induction of CYP1A1 and CYP1A2 enzyme activity. The relative potency differs by an order of magnitude in female mice following subchronic exposure to 2,3,3′,4,4′-PenCB (IUPAC No. 105) (DeVito et al., 2000). Neither spleen weight nor thymus weight changes, but the liver weight is significantly increased by 2,2′,4,4′,5,5′-HexCB treatment in pregnant mice (Mattsson et al., 1981).

The TEQ of 2,3,4,7,8-PeCDF showed significant differences for the *CYP1A1* (rs4646903) genotypes. The pharmacokinetics of 2,3,4,7,8-PeCDF have been studied in humans. In Yucheng patients in Taiwan who had been exposed to high levels of 2,3,4,7,8-PeCDF, this dioxin was the greatest contributor to the toxic effects because it accounted for 70% of the total dioxin TEQ in maternal blood (Masuda, 2001). Matsueda et al. (2007) examined the dioxin levels and congener distributions in blood samples of Yusho patients in Japan and normal controls, especially in relation to their respective exposure routes. They reported that the absorptivity and rate of metabolism and elimination for dioxin congeners depend on the exposure source. Further work is needed to confirm these findings for *AHR* and *CYP1A1* in dioxin congener studies in humans, especially in pregnant women, because chronic exposure to low levels of 2,3′,4,4′,5-PenCB (IUPAC No. 118), 2,3,3′,4,4′-PenCB (IUPAC No. 105), 2,3′,4,4′,5,5′-HexCB (IUPAC No. 167), and 2,3,4,7,8-PeCDF in the environment could be causally confirmed by epidemiological studies.

Although genetic polymorphisms cannot be changed, adverse health effects of dioxins could be prevented by modulating exposure levels, especially among individuals with increased genetic susceptibility, because dioxins may be a modifiable environmental pollutant. For example, one way to reduce dioxin exposure in

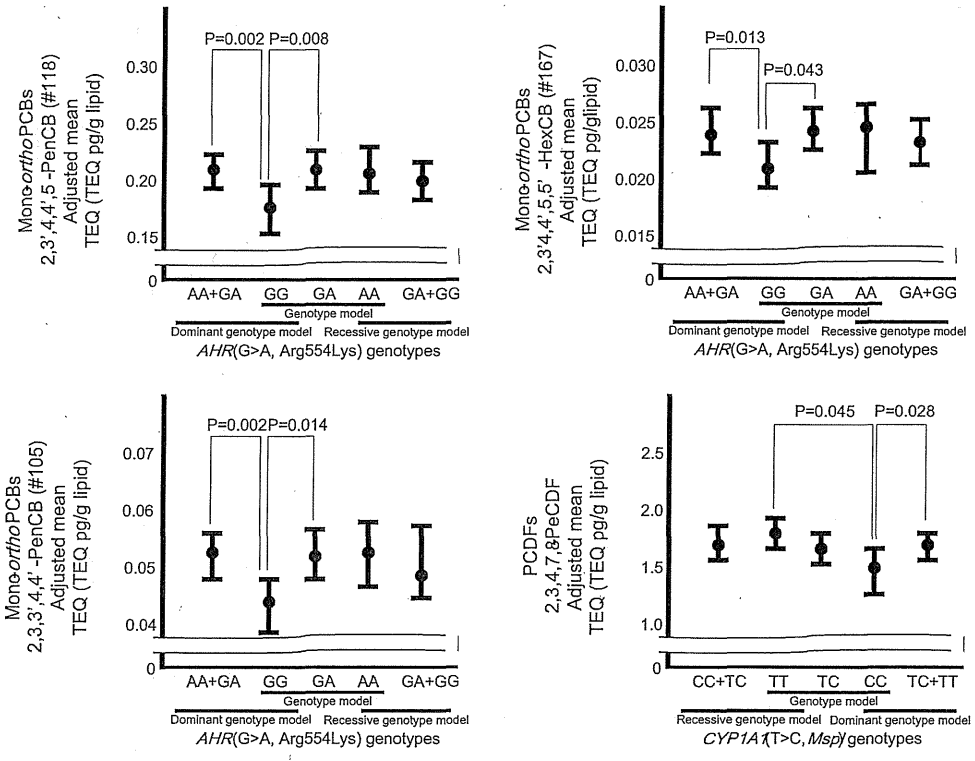

Fig. 2. TEQs for 2,3′,4,4′,5-PenCB (IUPAC No. 118), 2,3,3′,4,4′,-PenCB (IUPAC No. 105), 2,3′,4,4′,5,5′-HexCB (IUPAC No. 167) and 2,3,4,7,8-PeCDF in the generalized linear model of dioxin congeners among *AHR* and *CYP1A1* polymorphisms of pregnant women in Sapporo, Hokkaido, Japan.
Dots and bars are the adjusted means and 95% confidence intervals, respectively. The means were adjusted for maternal age, maternal height, maternal weight before pregnancy, caffeine intake during pregnancy, alcohol consumption during pregnancy, parity, maternal smoking status during pregnancy, maternal educational level, annual household income, inshore fish intake during pregnancy, deep-sea fish intake during pregnancy, and blood sampling period in the generalized linear model.

pregnant women is to minimize consumption of inshore fishes such as horse mackerels and sardines, which contain large quantities of dioxin.

The main strength of this study is that the dioxin concentrations were very accurate because we used highly sensitive methods for dioxin measurement. The present study also has a few limitations. First, we did not measure any metabolites of dioxins or placental AHR and CYP1A1 activity. Some metabolites are produced from one dioxin congener, and distinguishing the metabolites from the congeners was difficult. Thus, we could not measure them. Second, the functional consequences of the Pro/Ala substitution in *AHRR* remain largely unknown. A novel human AHRR complementary DNA that lacks the exon with the Pro185Ala polymorphism represses AHR (Karchner et al., 2009), but further studies are needed to confirm whether this mutation has any functional consequences.

In the present study, differences in dioxin blood concentrations were relatively low. Despite this, partial differences in health effects may exist, depending on the genetic polymorphism. Consequently, further longitudinal cohort studies should be carried out to confirm our findings. Moreover, further studies are also needed to investigate the effects of dioxins on developing school-age children. We are currently following the children of the mother–infant pairs in our study up to school age to determine whether exposure to low levels of dioxins during gestation affects their neurodevelopment, growth or risk of developing allergies. The results are forthcoming. We will also focus our attention not only on dioxin-metabolizing genes but also on the effects of polymorphisms on sex hormone production. Additional molecular and genetic epidemiological studies are needed to further elucidate the effects of both environmental and genetic factors in humans in the current and subsequent generations.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Original Article

Effects of Maternal 5,10-Methylenetetrahydrofolate Reductase C677T and A1298C Polymorphisms and Tobacco Smoking on Infant Birth Weight in a Japanese Population

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ABSTRACT

Background: Intracellular folate hemostasis depends on the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene. Because 5,10-*MTHFR* 677TT homozygosity and tobacco smoking are associated with low folate status, we tested the hypothesis that smoking in mothers with 5,10-*MTHFR* C677T or A1298C polymorphisms would be independently associated with lower birth weight among their offspring.

Methods: We assessed 1784 native Japanese mother-child pairs drawn from the ongoing birth cohort of The Hokkaido Study on Environment and Children's Health. Data (demographic information, hospital birth records, and biological specimens) were extracted from recruitments that took place during the period from February 2003 to March 2006. Maternal serum folate were assayed by chemiluminescent immunoassay, and genotyping of 5,10-*MTHFR* C677T/A1298C polymorphisms was done using a TaqMan allelic discrimination assay.

Results: The prevalence of folate deficiency (<6.8 nmol/L) was 0.3%. The 5,10-*MTHFR* 677CT genotype was independently associated with an increase of 36.40 g (95% CI: 2.60 to 70.30, $P = 0.035$) in mean infant birth weight and an increase of 90.70 g (95% CI: 6.00 to 175.50, $P = 0.036$) among male infants of nonsmokers. Female infants of 677TT homozygous passive smokers were 99.00 g (95% CI: −190.26 to −7.56, $P = 0.034$) lighter. The birth weight of the offspring of smokers with 5,10-*MTHFR* 1298AA homozygosity was lower by 107.00 g (95% CI: −180.00 to −33.90, $P = 0.004$).

Conclusions: The results suggest that, in this population, maternal 5,10-*MTHFR* C677T polymorphism, but not the 5,10-*MTHFR* A1298C variant, is independently associated with improvement in infant birth weight, especially among nonsmokers. However, 5,10-*MTHFR* 1298AA might be associated with folate impairment and could interact with tobacco smoke to further decrease birth weight.

Key words: birth weight; tobacco smoking; *MTHFR* SNPs; folate; Japan

INTRODUCTION

Intracellular folate hemostasis depends on the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene, which is located at position 36 on the short arm of chromosome 1. This gene codes for the enzyme *MTHFR*, which catalyses the irreversible conversion of 5,10-*MTHFR* to 5-methyltetrahydrofolate, a substrate for methylation of homocysteine to methionine. Thus far, 14 rare mutations in *MTHFR* have been described, but the 2 most common single nucleotide polymorphisms (SNPs) are 5,10-*MTHFR* C677T (dbSNP ID: rs1801133)—a missense mutation in exon 4, characterized by an alanine to valine substitution on codon 222—and 5,10-*MTHFR* A1298C (dbSNP ID: rs1801131)—a point mutation in exon 7 characterized by a glutamate to alanine substitution on codon 429.[1] 5,10-*MTHFR* C677T is located in the catalytic N-terminal domain of the enzyme, while 5,10-*MTHFR* A1298C is located in the regulatory domain of the enzyme.[2]

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Biochemically, the 5,10-*MTHFR* C677T polymorphism is associated with thermolability and reduced enzyme activity. The metabolic consequences are folate deficiency and mild hyperhomocysteinemia, a risk factor for thrombotic vascular diseases. It has been suggested that oxidative stress, platelet aggregation, and endothelial cell dysfunction contribute to the vasculotoxicity of homocysteine, and 5,10-*MTHFR* polymorphisms have been widely investigated in relation to a spectrum of several disease outcomes. Specifically, several studies have identified maternal 5,10-*MTHFR* C677T polymorphisms as obstetric genetic risk factors for spina bifida, placenta-related vasculopathies, spontaneous fetal loss, preterm delivery (PTD), low birth weight (LBW), small for gestational age (SGA), neurodevelopmental delays, and other congenital anomalies.[3–15] However, several other investigators have found no such associations.[16–25] This confusion might be explained by the fact that phenotypic expression of this genetic trait depends on folate status and other environmental factors that vary by geographic region and race.

Although the functional consequences of the 5,10-*MTHFR* A1298C variant are not well known, it is a risk factor for neural tube defects,[26,27] and compound heterozygosity (5,10-*MTHFR* 677CT/1298AC) has been reported to have a biochemical profile similar to that of 677TT homozygosity.[1,28]

Maternal smoking during pregnancy is an established risk factor for intrauterine growth retardation (IUGR), SGA, PTD, and other adverse pregnancy outcomes.[29] More recently, smoking has been associated with nutritional deficiencies, including folate deficiency.[30–32] LBW secondary to IUGR or PTD remains a public health concern because it increases the risk of morbidity and mortality throughout life.

In Japan, there have been genetic association studies of the relation between folate and cardiovascular pathologies, cancers, *Helicobacter pylori* infection, and periodontal diseases. However, only a few such studies have investigated obstetric events, and none has considered infant birth size.[3,6,17,33–35] We therefore tested the hypothesis that maternal smoking in the presence of the 5,10-*MTHFR* C677T or A1298C polymorphisms independently reduces birth weight.

METHODS

Study design and participants

The study participants were native Japanese mother-child pairs drawn from an ongoing birth cohort: The Hokkaido Study on Environment and Children's Health. This ongoing cohort started in February 2003, and the details of the study have been previously described.[36] Briefly, all indigenous Japanese women who reserved antenatal care at any of 37 participating hospitals within Hokkaido during their first trimester of pregnancy were considered eligible. Health care personnel introduced the study, after which each potential participant was given an invitation that included a consent form, baseline questionnaire, and self-addressed envelopes for return of the signed consent forms and completed questionnaires. The participants were recruited between February 2003 and March 2006. Only participants with linked and integrated data (5772; 61.8%) were included in the baseline population of this study. The response rate for each variable was at least 70.0% from various sources. Based on the population allele frequencies of the 5,10-*MTHFR* C677T[37] and A1298C[38] polymorphic variants specific to Japanese and the prevalence of tobacco smoking during pregnancy,[39] minimum sample sizes were calculated by using genetic software.[40] We randomly selected 1805 extracted genomic DNAs, attempted to discriminate the alleles of 5,10-*MTHFR* polymorphisms, and successfully genotyped 1784, which were ultimately used in the data analysis (Figure 1). The Institutional Ethical Board for Human Gene and Genome Studies of Hokkaido University Graduate School of Medicine approved the study protocol.

Methods of data collection

Data were acquired from baseline self-administered questionnaires, hospital records of infant births, and postpartum self-administered questionnaires. Baseline information included biodata, lifestyle habits, drugs (including use of nutritional supplements), and gynecologic and obstetric histories. Infant birth records from hospitals had information about birth weight, gestational age at delivery, sex, obstetric events during index pregnancy, and congenital anomalies, among other information. Postpartum questionnaires collected information on infant anthropometric parameters, active or passive tobacco smoking during the index pregnancy, and whether the index pregnancy was eventful. Each variable in the dataset had a response rate of at least 70.0%, although the item on smoking status decreased from 99.0% at baseline to 70.0% after pregnancy. Whole-blood specimens were collected during the first trimester for serum folate assays; subsequent collections of whole blood specimens were stored at −80°C for genetic analyses.

Folate assay

Serum folate was assayed by a commercial laboratory (SRL, Inc. Tokyo, Japan) using an automated competitive protein binding (CPB) chemiluminescent enzyme immunoassay (CLEIA) technique according to the manufacturer's protocols. This type of assay has an intra- and inter-assay imprecision of 10.0% or less and has become common in in vitro studies, as it is less costly, faster, and convenient. In addition, the need for smaller samples is advantageous in large scale epidemiologic studies.[41] The specific assay method for this study was the ADVIA Centaur technique, which has a coefficient of variation between 4.0% to 4.3%.[42] Analyses were conducted in batches, which were scheduled with regard to recruitment period and laboratory procedure.

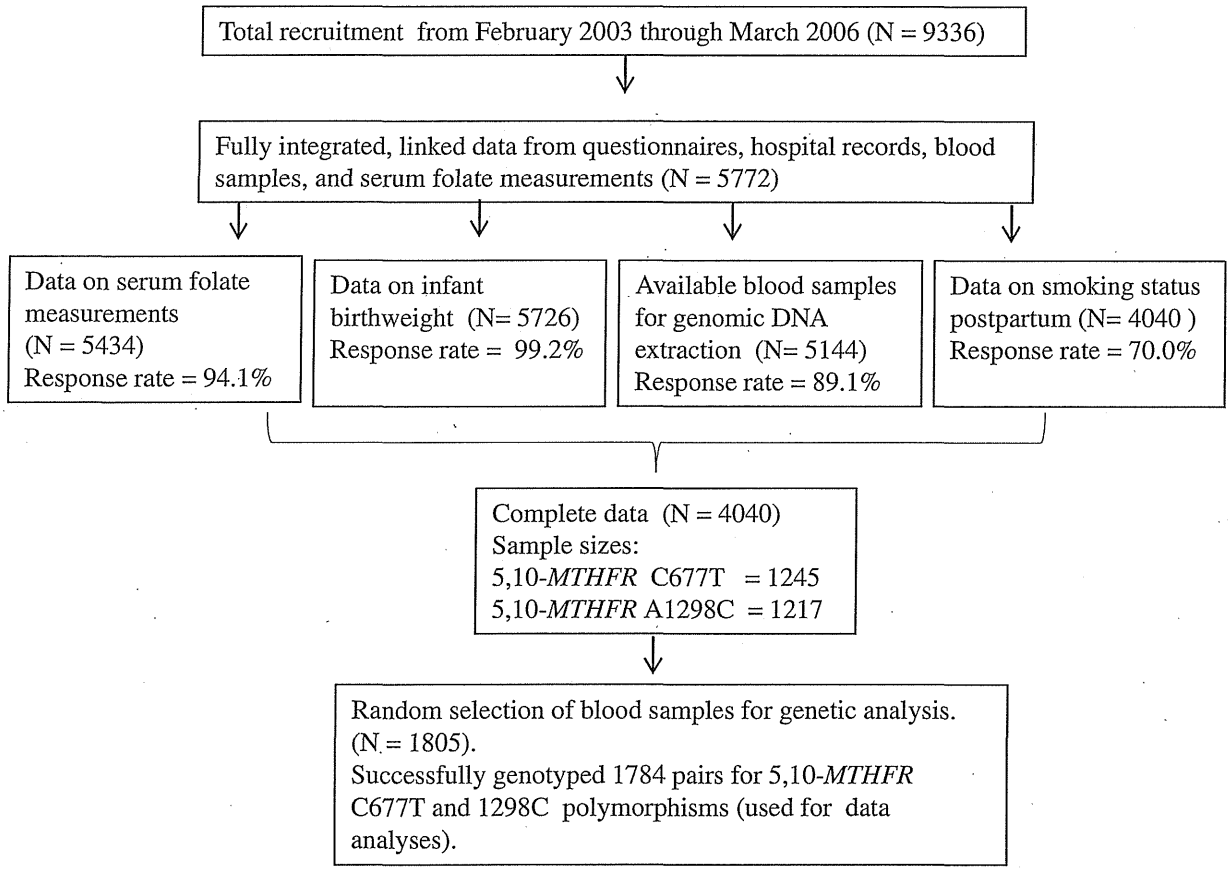

Figure 1. Study selection flow chart

Selection and genotyping of single nucleotide polymorphisms (SNPs)

We chose the 2 most common SNPs of this gene, namely C677T and A1298C, which have minor allele frequencies (MAF) of 35.2%[37] and 19.0%,[38] respectively, among Japanese. Genomic DNAs were extracted using a Maxwell 16 Instrument (Promega Corporation, WI, USA). DNA amplifications were performed in batches on 96-well micro-amp reaction plates using validated TaqMan probes for *MTHFR* C677T and A1298C (assay IDs: C_1202883_20 and C_850486_20), respectively, on a Gene Amp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) with an end-point allelic discrimination (AD) assay on a 7300/7500 Real-time PCR System[43] (Applied Biosystems, Foster City, CA, USA). We randomly selected 95 samples (5.0% of the successfully genotyped samples) and repeated genotyping to check for genotyping quality. The results were 100% concordant.

Definition of variables

Environmental exposures

Overall smoking status was classified into 3 categories using both self-reported active smoking and passive exposure to environmental tobacco smoke (ETS) at home. Nonsmokers had no history of active smoking or exposure to ETS at home. Nonsmokers and quitters with ETS exposure were classified as the passive smoking group, while smokers consisted of active smokers irrespective of ETS exposure status. Quitters with no ETS exposure during the first trimester had mean infant birth weights similar to those of nonsmokers; hence, they were added to the nonsmoking group. Mothers who quit during the second or third trimesters were added to the active smoking group.

Genetic exposures

The 5,10-*MTHFR* C677T and A1298C genotypes were categorized as dominant homozygous, heterozygous, and recessive homozygous genotypes (677CC, 677CT, and 677TT; and 1298AA, 1298AC, and 1298CC, respectively).

Statistical analyses

Univariate ANOVA with multiple comparison tests was performed to assess the main effects of maternal 5,10-*MTHFR* C677T and A1298C polymorphisms and smoking on serum folate levels, while ANCOVA was used to investigate the interactive association between smoking and 5,10-*MTHFR* C677T and A1298C polymorphisms in relation to folate status and infant birth weight. Serum folate was log-transformed before the analyses and back-transformed after the analyses. Known major predictors of infant birth weight (infant sex, gestational age at delivery, maternal age, maternal

prepregnancy weight, maternal height, parity, and alcohol intake during pregnancy) were adjusted for in the multivariate regression analyses. Use of a folic acid supplement, which was highly correlated with serum folate levels, was also included as a covariate. Smoking status was adjusted for when we assessed the predictive power of each SNP on birth weight. Categorical covariates were dichotomized to fit the regression equation. Subgroups with few participants (ie, subgroups for the 1298CC genotype) were excluded from the regression analyses. We used the codominant genetic model and per-allele approach. Our preliminary analyses revealed that mean serum folate was highest for the *MTHFR* 1298AC genotype. Because adequate folate status is an integral part of our hypothesis, we decided that it was biologically plausible to set 1298AC as the reference category in the regression analyses. Predictors were entered simultaneously into the equation. Assessment of the 5,10-*MTHFR* C677T and A1298C genotypes for deviation from the Hardy-Weinberg equilibrium, and other evaluations of data quality, were conducted using Haploview version 4.2 software.[44] All other analyses were performed using SPSS version 16.00 for Windows (SPSS Inc., Chicago, IL, USA). The level of statistical significance was set at less than 0.05.

RESULTS

The maternal mean serum folate (SD) level was 16.4 (1.5) nmol/L. The prevalence of folate deficiency (<6.8 nmol/L) was 0.3%; most (73.0%) mothers had adequate folate status (≥13.6 nmol/L). The prevalence of folic acid supplementation was 10.0%. Mean infant birth weight (SD) was 3040 (374) g. The prevalence of active smoking during pregnancy was 15.9%, while that of passive smoking was 53.0%. The distributions of the 5,10-*MTHFR* C677T and A1298C genotypes did not deviate from the Hardy-Weinberg equilibrium ($P = 0.546$ and 0.909, respectively). The frequencies of *MTHFR* 677CC, 677CT, and 677TT were 37.3%, 46.7%, and 16.0%, respectively, while those of *MTHFR* 1298AA, 1298AC, and 1298CC were 62.7%, 33.1%, and 4.2%, respectively. A strong LD ($D' = 0.943$) between *MTHFR* C677T and A1298C was also observed, and the minor allele frequencies were 0.392 and 0.205, respectively (Table 1). These findings were similar to those of previous studies of Japanese populations.[6,37,38,45–47] We used 2-way analysis of variance to assess the main effects of 5,10-*MTHFR* on maternal mean serum folate concentration. Carrying the T allele was associated with a decrease in mean serum folate level, and the lowest level (14.1 nmol/L) was observed in the 677TT homozygous group. Tukey's honestly significant differences (HSD) of 1.0 nmol/L ($P = 0.008$) and 3.8 nmol/L ($P < 0.001$) were observed between 677CC versus 677CT and between 677CC versus 677TT, respectively. In contrast, carrying the 1298C allele was associated with higher mean serum folate levels. A Tukey's HSD of 1.5 nmol/L ($P < 0.001$) was observed between 1298AA versus 1298AC (Figure 2). Mean serum folate levels in the analysis of covariance were generally lower among smokers for all 5,10-*MTHFR* C677T genotypes, and the lowest level was found among 677TT homozygotes (11.8 nmol/L, $P_{interaction} < 0.001$). With regard to 5,10-*MTHFR* A1298C genotypes, the lowest mean folate level was observed among smokers with the 1298AA homozygous genotype, ($P_{interaction} < 0.001$; Figure 3).

We initially explored the role of maternal serum folate during the first trimester as a predictor of infant birth weight and found no significant linear association. After stratification by folate status, low folate status (<13.6 nmol/L) was associated with a nonsignificant reduction in birth weight. After stratification by birth weight status, low folate status was associated with a 34.00 g ($P = 0.045$) reduction in mean birth weight of infants in the normal birth weight group (data not shown).

To investigate whether the maternal 5,10-*MTHFR* C677T and A1298C polymorphisms were independently associated with birth weight, we conducted a multiple regression analysis with adjustments for known major predictors of birth weight.

Table 1. Characteristics of 1784 mother-child pairs

Characteristic	*n* (%)
Maternal age (years)	30.0 (4.3)[e]
Maternal height (cm)	158.0 (5.1)[e]
Prepregnancy weight (kg)	53.0 (9.3)[e]
Maternal serum folate (nmol/L)	16.4 (1.5)[e]
Gestational age at delivery (weeks)	38.9 (1.3)[e]
Infant birth weight (g)	3040 (374)[e]
Infant sex	
Male	873 (48.9)
Female	911 (51.1)
Parity	
Nulliparous	391 (21.9)
Parous	1393 (78.1)
Alcohol intake during pregnancy	
No	1499 (84.0)
Yes	285 (16.0)
Tobacco smoking during pregnancy	
Nonsmoker	555 (31.1)
Passive smoker	946 (53.0)
Smoker	283 (15.9)
Folic acid supplementation	
No	1601 (89.7)
Yes	183 (10.3)
Maternal *MTHFR* C677T genotype[a,b]	
CC	666 (37.3)
CT	833 (46.7)
TT	285 (16.0)
CT/TT	1118 (62.7)
Maternal *MTHFR* A1298C genotype[c,d]	
AA	1118 (62.7)
AC	591 (33.1)
CC	75 (4.2)
AC/CC	666 (37.3)

[a]HWE = Hardy-Weinberg equilibrium $P = 0.5463$
[b]MAF = Minor allele frequency = 0.392
[c]HWE = Hardy-Weinberg equilibrium $P = 0.9091$
[d]MAF = Minor allele frequency = 0.205
[e]Mean (SD) *MTHFR* = Methylenetetrahydrofolate reductase gene

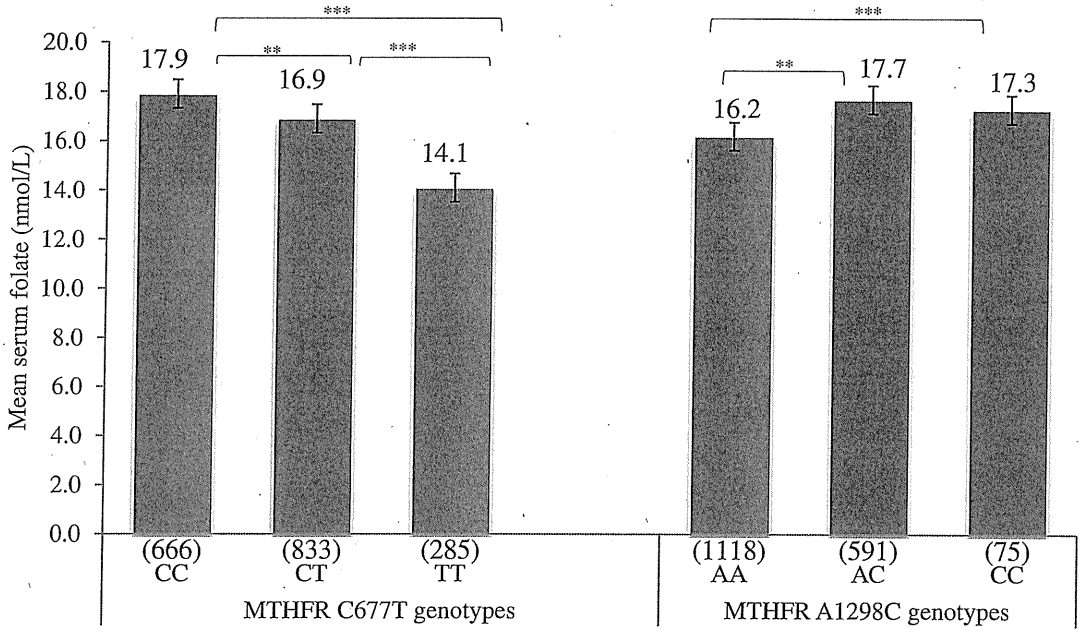

Figure 2. Maternal mean serum folate levels across *MTHFR* C677T and A1298C genotypes.
$^{**}P < 0.01$, $^{***}P < 0.001$ Univariate analysis with Tukey's honestly significant differences test. Values in parentheses are counts in each group.

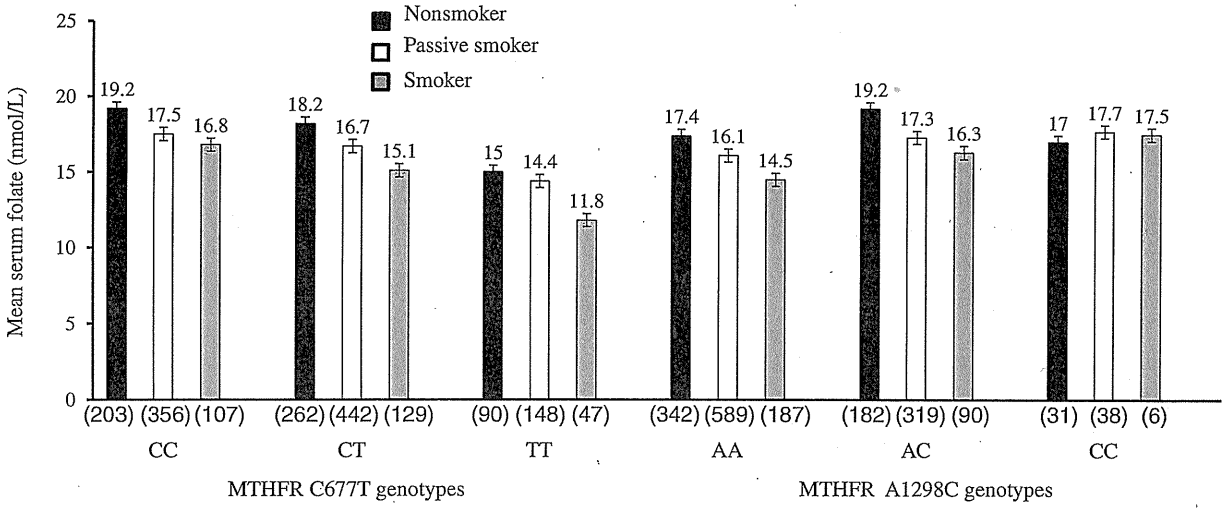

Figure 3. Maternal mean serum folate levels across *MTHFR* C677T and A1298C genotypes according to smoking status. ANCOVA $P_{interaction} < 0.001$. For MTHFR C677T and A1298C. Values in parentheses are counts in each group.

Among infants of 677CT heterozygous mothers, adjusted mean birth weight was highest (3061 g) for 5,10-*MTHFR* C677T. The 677CT genotype was associated with a 36.40 g increase in mean infant birth weight (95% CI: 2.60 to 70.30, $P = 0.035$). Carrying the 677T allele was associated with a marginally significant 27.00 g increase in infant birth weight (95% CI: −3.76 to −59.47, $P = 0.084$).

Polymorphism in 5,10-*MTHFR* A1298C or carrying the 1298C allele was not significantly independently associated with birth weight, although the adjusted mean infant birth weight was highest (3048 g) in the 1298AC heterozygous group. The adjusted mean birth weight of infants of active tobacco smokers was lowest (2978 g) and was 85.00 g (95% CI: 133.30 to −36.80, $P = 0.001$) less than that of children born to nonsmokers (Table 2).

We stratified mothers by tobacco smoking status. Among nonsmokers, male infants of 677CT genotype mothers were 90.00 g (95% CI: −2.11 to 182.50, $P = 0.05$) heavier than the infants in the reference category, which was marginally statistically significant, while among passive smokers, female

Table 2. Association of maternal 5,10-*MTHFR* C677T, A1298C genotypes and tobacco smoking with infant birth weight (*N* = 1784)

Maternal 5,10-*MTHFR* polymorphisms/smoking status	*n*	Adjusted mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g	P_{trend}[c]
[a]*MTHFR* C677T genotype				
CC	666	3024.70 (14.51)	Reference	
CT	833	3061.13 (12.98)	36.40 (17.30) [2.60, 70.30]*	
TT	285	3015.15 (22.75)	4.00 (23.50) [−42.20, 50.10]	0.07
[a]*MTHFR* C677T allele				
C	2165	3044.97 (9.68)	Reference	
T	1403	3049.52 (11.29)	27.86 (16.12) [−3.76, 59.47]†	
[a]*MTHFR* A1298C genotype				
AA	1118	3036.58 (11.17)	Reference	
AC	591	3048.71 (15.67)	14.47 (16.78) [−18.45, 47.38]	
CC	75	3028.59 (44.69)	−27.65 (39.36) [−104.85, 49.55]	0.49
[a]*MTHFR* A1298C allele				
A	2827	3040.77 (9.1)	Reference	
C	741	3046.43 (14.78)	9.74 (16.16) [−21.95, 41.43]	
[b]Smoking status				
Nonsmoker	555	3062.81 (14.11)	Reference	
Passive smoker	946	3046.08 (10.70)	−14.40 (17.90) [−49.50, 20.60]	
Smoker	283	2978.29 (19.70)	−85.01 (24.60) [−133.30, −36.80]**	0.001

**$P < 0.01$. *$P < 0.05$. †$P < 0.1$. Δ (Change in mean birth weight) B (Unstandardized coefficients) SE (standard error). (CI) confidence interval. (*MTHFR*) Methylenetetrahydrofolate reductase. Grams (g). [a]Multiple linear regression adjusted for gestational age, infant sex, maternal age, prepregnancy weight, height, parity, smoking during pregnancy, alcohol intake during pregnancy, and folic acid supplement use. [b]Multiple linear regression adjusted for gestational age, infant sex, maternal age, prepregnancy weight, height, parity, and folic acid supplement intake. [c]Polynomial univariate analysis.

infants of 677TT homozygous mothers were 99.00 g (95% CI: −190.26 to −7.56, $P = 0.03$) lighter than reference. None of the minor-allele genotypes among smokers showed any significant effect on infant birth weight as compared with those in the major-allele genotypes (data not shown). Per-allele analyses revealed that carrying the 677T allele was associated with a 68.00 g (95% CI: −121.74 to −15.27, $P = 0.012$, $P_{trend} = 0.003$) reduction in mean birth weight among infants of smokers and an 89.00 g (95% CI: −168.89 to −9.56, $P = 0.028$, $P_{trend} = 0.018$) reduction among female infants (Table 3a). Furthermore, smoking in mothers carrying the 1298A allele was associated with a 92.00 g (95% CI: −144.46 to −40.96, $P < 0.001$, $P_{trend} = 0.091$) reduction in mean birth weight. Males were lighter by 79.00 g (95% CI −150.73 to −8.58, $P = 0.028$, $P_{trend} = 0.228$), while females were lighter by 107.00 g (95% CI: −182.78 to −31.54, $P = 0.006$, $P_{trend} = 0.112$; Table 3b).

In cross-classification interactive analyses, infants born to nonsmokers with 5,10-*MTHFR* 677CT genotypes had the highest mean birth weight (3092 g); male newborns were 90.70 g (95% CI: 6.00 to 175.50, $P = 0.036$) heavier than the male infants of nonsmoking 677CC mothers, ($P_{interaction} = 0.020$; Table 3a). The 5,10-*MTHFR* 1298AA genotype was associated with a 107.00-g (95% CI: −165.67 to −47.52, $P < 0.001$) decrease in mean infant birth weight among smokers. Stratification by infant sex did not yield obvious differences in birth weight, $P_{interaction} = 0.040$; Table 3b). When 1298AC was set as the reference category, the 5,10-*MTHFR* 1298AA genotype was associated with a 107.00-g (95% CI, −180.00 to −33.90, $P = 0.004$) decrease in mean infant birth weight in smokers; the effect was more obvious in male infants (117.00 g; 95% CI: −218.60 to −14.70, $P = 0.025$; data not shown).

DISCUSSION

Among nonsmokers, we found an association of maternal 5,10-*MTHFR* 677CT heterozygosity with higher infant birth weight, while 5,10-*MTHFR* 677TT homozygosity was associated with lower birth weight among female infants of passive tobacco smokers. In addition, among smokers, 5,10-*MTHFR* 1298AA homozygosity was associated with low folate status and lower birth weight. To our knowledge, this is the first study to report such findings for a Japanese population.

Maternal 5,10-*MTHFR* C677T, *MTHFR* A1298C and serum folate status

Our results showed an association between the 5,10-*MTHFR* 677T allele and low folate status, which agrees with the findings of earlier reports.[6,46] 677TT homozygosity was associated with low folate status, and values were much lower among active and passive smokers, which suggests independent and combined effects of tobacco smoke and 5,10-*MTHFR* C677T polymorphism on folate status.

In contrast, the 5,10-*MTHFR* 1298C allele was associated with higher serum folate levels, while the 1298AA genotype was associated with lower folate levels. Although the metabolic and clinical functions of this SNP have not been fully characterized, it is currently being studied by a number of investigators. In a recent study of Koreans, mean plasma

Table 3a. Association of maternal 5,10-*MTHFR* C677T polymorphism and tobacco smoking with infant birth weight (*N* = 1784)

5,10-*MTHFR*	Smoking status	Overall (*N* = 1784)			Males (*N* = 873)			Females (*N* = 911)		
		n	Adjusted Mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g	*n*	Adjusted Mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g	*n*	Adjusted Mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g
C677T genotype										
CC	Nonsmoker	203	3008.84 (25.84)	Reference	93	3080.01 (37.75)	Reference	110	2949.95 (34.58)	Reference
	Passive smoker	356	3035.93 (19.85)	9.5 (29.3) [−47.9, 66.9]	187	3037.8 (27.16)	−39.6 (40.5) [−119.2, 39.9]	169	3033.88 (29.16)	59.3 (41.6) [−22.3, 140.8]
	Smoker	107	3017.29 (37.52)	−38.7 (39.8) [−116.8, 39.4]	52	3109.23 (52.51)	−37.7 (55.8) [−147.3, 71.8]	55	2928.76 (51.18)	55.3 (56.5) [−166.2, 55.6]
CT	Nonsmoker	262	3092.43 (24.44)	60.0 (31.0) [−0.9, 120.9]†	133	3162.77 (35.43)	90.7 (43.20) [6.0, 175.50]*	129	3020.99 (32.59)	34.1 (44.1) [−52.4, 120.6]
	Passive smoker	442	3068.18 (17.15)	47.5 (28.2) [−7.9, 102.9]	198	3114.72 (24.29)	21.2 (40.3) [−57.8, 100.3]	244	3030.45 (23.78)	62.1 (39.1) [−14.6, 138.8]
	Smoker	129	2973.99 (32.43)	−53.1 (37.8) [−127.2, 21.0]	70	3041.21 (35.16)	−39.8 (51.1) [−140.0, 60.4]	59	2894.24 (55.93)	−63.5 (55.2) [−171.9, 44.9]
TT	Nonsmoker	90	3087.77 (38.76)	59.1 (42.0) [−23.3, 141.4]	43	3123.57 (54.59)	15.1 (58.8) [−100.6, 130.5]	47	3055.09 (55.04)	91.7 (59.0) [−24.0, 207.4]
	Passive smoker	148	2984.38 (31.57)	−14.4 (36.1) [−85.2, 56.3]	74	3045.41 (45.87)	11.1 (50.1) [−87.2, 109.4]	74	2922.5 (42.44)	−39.7 (51.1) [−140.0, 60.6]
	Smoker	47	2974.11 (58.61)	−49.1 (53.9) [−154.8, 56.7]	23	3067.13 (73.23)	−53.7 (74.9) [−200.7, 93.3]	24	2884.96 (88.5)	−48.3 (76.7) [−198.8, 102.2]
	$P_{interaction}$			0.03			0.02			0.14
C677T allele[d]										
C	Nonsmoker	465	3059.64 (15.39)	Reference	226	3137.62 (21.58)	Reference	239	2987.47 (21.95)	Reference
	Passive smoker	798	3053.06 (11.64)	−13.37 (19.69) [−51.98, 25.25]	385	3075.26 (56.28)	−60.94 (26.60) [−113.15, −8.73]*	413	3030.17 (16.61)	35.80 (29.20) [−21.51, 93.11]
	Smoker	236	2979.64 (21.57)	−63.45 (33.22) [−128.61, 1.71]†	122	3046.22 (29.14)	−57.52 (46.52) [−148.82, 33.78]	114	2909.50 (32.18)	−78.85 (47.97) [−173.00, 15.30]
	$P_{interaction}$			0.45			0.75			0.06
T	Nonsmoker	352	3084.10 (17.66)	Reference	176	3159.40 (24.30)	Reference	176	3015.04 (25.68)	Reference
	Passive smoker	590	3055.28 (13.57)	−9.93 (17.12) [−23.65, 43.52]	272	3103.07 (19.34)	12.38 (24.16) [−35.03, 59.79]	318	3009.48 (19.08)	7.75 (24.40) [−40.15, 55.64]
	Smoker	176	2975.05 (24.89)	−68.50 (27.14) [−121.74, −15.27]*	93	3042.31 (33.20)	−48.48 (36.51) [−120.13, 23.18]	83	2909.60 (37.38)	−89.22 (40.59) [−168.89, −9.56]*
	$P_{interaction}$			0.34			0.48			0.46

***$P < 0.001$. *$P < 0.05$. †$P < 0.1$. Δ (Change in mean birth weight), B (Unstandardized coefficient) SE (Standard error). CI (Confidence interval), g (Grams). [a]Multiple linear regression adjusted for infant sex, gestational age at delivery, maternal age, maternal height, prepregnancy weight, parity, alcohol intake during pregnancy, and folic acid supplement intake. [b]Multiple linear regression adjusted for gestational age at delivery, maternal age, maternal height, prepregnancy weight, parity, alcohol intake during pregnancy, and folic acid supplement intake. [c]Excluded from the regression analyses. [d]P_{trend} by smoking status (C allele = 0.021, Males = 0.045, Females = 0.040 and T allele = 0.003, males = 0.073, Females = 0.018).

homocysteine was higher among 1298AA homozygotes as compared with those carrying the 1298C allele.[48] Because serum folate is inversely correlated with plasma homocysteine, we inferred that our study population might have a similar plasma homocysteine distribution across genotypes. A report from Portugal noted that 1298AC heterozygosity was associated with a high plasma folate level and that the level was lowest among 1298CC homozygotes,[49] which is similar to the findings of the present study. Our findings contradict those of a study of a Dutch population, in which *MTHFR* A1298C alone was not associated with any biochemical abnormalities except when in combination with *MTHFR* C677T, specifically compound heterozygosity 5,10-*MTHFR* 677CT/1298AC.[28] The fact that our findings were similar to those from a report on a Korean population is genetically plausible because racial, geographic, and nutritional disparities might account for differences in the functional characteristics of 5,10-*MTHFR* SNPs.[50,51]

Effects of maternal 5,10-*MTHFR* A1298C polymorphism and tobacco smoke on infant birth weight

Smokers carrying 1298A alleles delivered infants with lower mean birth weights, especially female infants; however, these results must be interpreted with caution because alleles do not act in isolation. The effect of the maternal 5,10-*MTHFR* 1298AA genotype in reducing the birth weight of infants delivered by tobacco smokers might be due to low folate status associated with the 1298AA genotype. Perhaps some essential folate-dependent cellular processes were compromised. Cells that lack folate have been observed to accumulate in the S-phase of the cell cycle. Such cells have higher uracil misincorporation and DNA damage,[52] which might have a role in the impairment of fetal growth. Moreover, chronic deficits in extracellular and intracellular folate due to the effects of tobacco smoke might have been severe enough to inflict nutritional stress. In our study, we could not examine the role of the 1298CC genotype among

Table 3b. Association of maternal 5,10-*MTHFR* A1298C polymorphism and tobacco smoking with infant birth weight (*N* = 1784)

5,10-*MTHFR*	Smoking status	Overall (*N* = 1784)			Males (*N* = 873)			Females (*N* = 911)		
		n	Adjusted Mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g	*n*	Adjusted Mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g	*n*	Adjusted Mean birth weight (SE) g	Adjusted Δ B (SE) [95% CI] g
A1298C genotype										
AA	Nonsmoker	342	3071.9 (20.47)	Reference	169	3145.63 (28.99)	Reference	173	3000.74 (27.94)	Reference
	Passive smoker	589	3036.45 (15.32)	−29.04 (22.36) [−72.91, 14.82]	266	3069.39 (23.03)	−59.35 (31.34) [−120.86, 2.16]	323	3009.36 (20.43)	−3.07 (32.08) [−66.03, 59.90]
	Smoker	187	2973.15 (26.53)	−106.59 (30.12) [−165.67, −47.52]***	94	3045.5 (31.69)	−104.19 (41.36) [−185.37, −23.03]*	93	2900.02 (41.47)	−113.45 (44.05) [−199.91, −26.99]*
AC	Nonsmoker	182	3058.19 (29.74)	−1.66 (30.08) [−60.66, 57.34]	83	3110.86 (45.78)	13.56 (42.70) [−70.26, 97.37]	99	3015.1 (38.68)	−13.88 (42.68) [−97.64, 69.89]
	Passive smoker	319	3049.36 (20.36)	−15.20 (25.59) [−65.39, 34.98]	173	3066.73 (25.49)	−51.98 (34.39) [−119.47, 15.51]	146	3028.88 (32.66)	21.20 (38.41) [−54.19, 96.59]
	Smoker	90	3027.25 (42.41)	−57.04 (39.11) [−133.74, 19.66]	46	3120.85 (51.65)	−59.18 (53.47) [−164.14, 45.78]	44	2927.12 (65.41)	−64.21 (58.00) [−178.05, 49.62]
CC	Nonsmoker	31	2959.81 (55.2)	−120.92 (61.81) [−242.16, 0.32]†	17	3037.65 (79.96)	−107.17 (81.49) [−267.12, 52.77]	14	2865.29 (69.02)	−142.72 (94.43) [−328.05, 42.61]
	Passive smoker	38	3092.97 (65.72)	13.12 (61.81) [−97.08, 123.31]	20	3158.9 (98.97)	14.55 (75.56) [−133.75, 162.84]	18	3019.72 (84.17)	6.64 (84.21) [−158.64, 171.91]
	Smoker	6[c]	2976.17 (247.92)	—	5[c]	3054.6 (288.05)	—	1[c]	2584	—
	$P_{interaction}$			0.04			0.03			0.22
A1298C allele[d]										
A	Nonsmoker	524	3069.05 (14.52)	Reference	252	3140.25 (20.45)	Reference	272	3004.92 (20.68)	Reference
	Passive smoker	908	3045.91 (10.93)	−15.55 (17.17) [−49.23, 18.12]	439	3075.06 (15.24)	−35.44 (24.30) [−83.13, 12.26]	469	3016.12 (15.67)	−6.65 (24.40) [−45.24, 50.54]
	Smoker	277	2978.20 (19.91)	−92.71 (26.38) [−144.46, −40.96]***	140	3043.18 (27.23)	−79.65 (36.21) [−150.73, −8.58]*	137	2912.57 (29.35)	−107.16 (38.53) [−182.78, −31.54]**
	$P_{interaction}$			0.15			0.22			0.47
C	Nonsmoker	213	3049.58 (22.66)	Reference	100	3124.26 (32.36)	Reference	113	2980.20 (31.83)	Reference
	Passive smoker	357	3056.17 (17.48)	−18.49 (19.73) [−20.21, 57.20]	193	3084.82 (23.06)	−4.28 (26.42) [−56.13, 47.58]	164	3031.86 (26.57)	41.38 (29.65) [−16.81, 99.57]
	Smoker	97	3009.71 (33.78)	−29.12 (34.99) [−97.75, 39.50]	51	3070.73 (44.90)	−19.37 (47.02) [−111.66, 72.92]	45	2942.56 (51.53)	−46.16 (52.88) [−149.95, 57.64]
	$P_{interaction}$			0.37			0.72			0.51

***$P < 0.001$. *$P < 0.05$. †$P < 0.1$. Δ (Change in mean birth weight). B (Unstandardized coefficient) SE (Standard error). CI (Confidence interval), g (Grams). [a]Multiple linear regression adjusted for infant sex, gestational age at delivery, maternal age, maternal height, prepregnancy weight, parity, alcohol intake during pregnancy, and folic acid supplement intake. [b]Multiple linear regression adjusted for gestational age at delivery, maternal age, maternal height, prepregnancy weight, parity, alcohol intake during pregnancy, and folic acid supplement intake. [c]Excluded from the regression analyses. [d]P_{trend} by smoking status (A allele = 0.091, Males = 0.228, Females = 0.112 and C allele = 0.752, males = 0.828, Females = 0.271).

smokers because of its low frequency. However, previous reports observed that the maternal 1298CC genotype was associated with a greater reduction in the risk of low birth weight as compared with the 1298AA genotype.[21] The 1298CC homozygous genotype was also reported to be protective against IUGR in Canadians.[53] Hyperhomocysteinemia might have increased the risk of placental vasculopathy via oxidative stress, endothelial cell dysfunction, and/or coagulopathies leading to feto-placental hypoperfusion.[5]

Adequate serum vitamin B_{12} status has been shown to decrease total plasma homocysteine levels in Japanese.[54] However, among smokers, the possible coexistence of nutritional deficiencies, including vitamin B_{12} deficiency, might have compromised the methylation of homocysteine to methionine, resulting in impaired fetal growth. The folate level was probably not adequate to silence the phenotypic expression of 1298AA among smokers. Higher exogenous folate may be needed to correct deficits and maintain ideal levels for optimal fetal growth.

With regard to the 5,10-*MTHFR* gene structure, the A1298C variant is located on the regulatory C-terminal domain, which contains protein retention signals that prevent delivery of proteins to the secretory pathway. It is possible that allosteric inhibitory interplay in the s-adenosyl methionine (SAM) and s-adenosyl homocysteine (SAH) cycle is involved in the functional behavior of this SNP in relation to folate status and mediation of fetal growth. Recently, the 5,10-*MTHFR* A1298C polymorphism was found to be associated with increased folate levels in red blood cells, in an inverse relationship with 5,10-*MTHFR* C677T polymorphism,[55] which suggests that both SNPs have different functional characteristics with regard to phenotypic expression.

Due to limited evidence on the functional role of the 5,10-*MTHFR* A1298C polymorphism, especially among Japanese,