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Title

Dioxin-metabolizing genes in relation to effects of prenatal dioxin levels and reduced birth size: The Hokkaido Study

Authors

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Highlights

- We examined the association of prenatal dioxins and polymorphism with birth size.
- Polymorphisms in 3 genes encoding dioxin-metabolizing enzymes were investigated.
- Polymorphisms were analyzed in 421 healthy pregnant Japanese women.
- Dioxin TEQ was associated with reduced birth weight reduction in the *GSTM1* null genotype.

Abbreviations

AHR, aromatic hydrocarbon receptor; CYP, cytochrome P450; CYP1A1, cytochrome P450 1A1; dbSNP, database single nucleotide polymorphism; *GSTM1*, glutathione S-transferase mu 1; HRGC/HRMS, high-resolution gas chromatography/high-resolution mass spectrometry; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; PenCB, pentachlorinated biphenyl; PenCDD, pentachlorinated dibenzo-*p*-dioxin; PenCDF, pentachlorinated dibenzofuran; TEQ, toxic equivalency; TetCDD, tetrachlorinated dibenzo-*p*-dioxin

Abstract

Objectives: We investigated the effects of maternal polymorphisms in 3 genes encoding dioxin-metabolizing enzymes in relation to prenatal dioxin levels on infant birth size in Japan.

Methods: We examined the relationship between dioxin exposure and birth size in relation to the polymorphisms in the genes encoding aromatic hydrocarbon receptor (*AHR* [G>A, Arg554Lys]), cytochrome P450 (CYP) 1A1 (T6235C), and glutathione S-transferase mu 1 (*GSTM1*; Non-null/null) in 421 participants using multiple linear regression models.

Results: In mothers carrying the *GSTM1* null genotype, a ten-fold increase in total dioxin toxic equivalency was correlated with a decrease in birth weight of -345 g (95% confidence interval: -584, -105).

Conclusions: We observed adverse effects of maternal *GSTM1* null genotype on birth weight in the presence of dioxins exposure during pregnancy.

Keywords

Dioxins, Pregnancy, Polymorphisms, Infants, Birth size

1. Introduction

Dioxins, such as polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and non-*ortho* and mono-*ortho* polychlorinated biphenyls (PCBs) are ubiquitous in the environment. They are produced in waste incineration and metal smelting and are side products of the synthesis of several chemicals, especially chlorophenoxy acid herbicides and hexachlorophene. Fish and meat consumption provides the main route for internal exposure to dioxins in the US and many European countries [1, 2, 3, 4]. In Japan, >90% of the total dioxin intake is dietary with the main sources being fish and other seafood [1, 5, 6]. The mean daily intake of total dioxins by the Japanese has been estimated as 3.22 pg toxic equivalency (TEQ)/kg body weight/day [6].

1,2,3,7,8-pentachlorinated dibenzo-*p*-dioxin (PenCDD), 2,3,4,7,8-pentachlorinated dibenzofuran (PenCDF), and 3,3',4,4',5-pentachlorinated biphenyl (PenCB) provide 63.1% of the total TEQ in Japanese [6]. Dioxins adversely affect human health via the aromatic hydrocarbon receptor (AHR) [7]. The toxic equivalency factor (TEF) values for 2,3,7,8-TetCDD, 1,2,3,7,8-PenCDD, and 2,3,4,7,8-PenCDF (1, 1, and 0.3, respectively) are larger than those of other congeners (0.0003-0.1) as indicated by a previous study [7]. We previously found the median body lipid levels of 2,3,7,8-TetCDD, 1,2,3,7,8-PeCDD, and 2,3,4,7,8-PenCDF to be 0.50, 3.93, and 5.54 pg TEQ/g lipid, respectively, in Japanese pregnant women [8]. The body levels of 1,2,3,7,8-PenCDD and 2,3,4,7,8-PenCDF are 10–100 times greater than those of other dioxin congeners in the placentas of Japanese nursing mothers [9].

Recent epidemiological studies suggested that the increased dioxin levels (PCDD mean: 16.01 TEQ pg/g lipid) in Japanese pregnant women affected by rice-bran oil disease (Yusho disease) are associated with an increased stillbirth rate [10], a reduced proportion of newborn males [10], and reduced birth size [11]. Increased dioxin levels have been associated

with reduced uterus size in 8-year-old girls (PCDD/PCDF median: 12 pg TEQ/g lipid) [12]. We observed that increased PCDD levels in Japanese pregnant women (median: 6.8 pg TEQ/g lipid) are associated with reduced birth size [13]. Polymorphisms in the genes encoding the aromatic hydrocarbon receptor (*AHR*) (G>A, Arg554Lys, dbSNP ID: rs2066853) and the cytochrome P450 (*CYP*) *1A1* (T6235C, dbSNP ID: rs4646903) have been associated with maternal dioxin levels during pregnancy [14]. An association between increased dioxin level during pregnancy (PCDD/PCDF mean: 11.75-11.76 pg TEQ/g lipid [15, 16] and PCDD/PCDF median: 13.8 pg TEQ/g lipid) and reduced infant birth size in Japanese women has been reported [15, 16, 17]. Moreover, increased dioxin levels in pregnant Japanese women (PCDD/PCDF median: 11.2 pg TEQ/g lipid) have been associated with neurodevelopmental behavior at 6 months of age [18]. Similarly, high dioxin levels during pregnancy (maternal PCDD/PCDF median: 6.64 pg TEQ/g lipid) correlated with behavior related to attention deficit–hyperactivity disorder in Dutch school-aged children [19].

Dioxins bind *AHR* and induce *CYP1A1* expression [20]. The absence of human glutathione S-transferase mu 1 (*GSTM1*; null genotype) is associated with increased induction of *CYP1A1* expression [21]. We have shown that *AHR* and *CYP1A1* polymorphisms are associated with greater maternal dioxin concentrations and/or TEQs [14]. Birth weight is lower in infants born to *GSTM1*-null smokers [22]. *CYP1A1* is well known to be involved in Phase I and *GSTM1* in Phase II of dioxin metabolism. Polymorphisms in *AHR*, *CYP1A1*, and *GSTM1* affect the expression or metabolic activity of their protein products [20, 23, 24]. These polymorphisms may mediate genetic susceptibility to maternal tobacco smoke exposure, and may be related to reduced birth sizes. Therefore, maternal *AHR*, *CYP1A1*, and *GSTM1* genotypes may also mediate genetic susceptibility to dioxins and may affect infant birth size. However, no reports have described maternal genetic susceptibility to low levels of

dioxins in relation to infant birth size.

Therefore, the objective of this study was to investigate maternal genetic polymorphisms in *AHR*, *CYP1A1*, and *GSTM1* that might affect the association between increased dioxin exposure during pregnancy and infant birth size among Japanese individuals.

2. Materials and Methods

2.1. Study population and data collection. We recruited 514 pregnant women between July 2002 and October 2005 from the Sapporo Toho Hospital in Sapporo, Japan (The Hokkaido Study on Environment and Children's Health) to participate in this study. Details of the population and data collection until delivery have been reported [25, 26]. Ten registered women were dropped from the study because of miscarriage, stillbirth, withdrawal before delivery, or drop-out at the beginning of the follow-up period. Participants with pregnancy-induced hypertension (N = 11), diabetes mellitus (N = 1), fetal heart failure (N = 1), and multiple births (N = 7) were excluded, resulting in a sample size of 484. In their last trimester, the participants completed a self-administered questionnaire on dietary habits (including inshore and deep-sea fish intake), smoking status, alcohol intake, caffeine intake, household income, educational level, and medical history. Dietary intake of inshore and deep-sea fish was ascertained from a frequency questionnaire, and was classified into five categories: almost every day, 3–4 times/week, 1–2 times/week, 1–2 times/month, or never. Participants were classified according to smoking status as follows: non-smokers defined as mothers who had never smoked or quit smoking until the first trimester, and smokers defined as mothers who continued smoking after their first trimester. For estimating caffeine and alcohol intake, we used the modified self-administered questionnaire described by Nagata et al [27, 28]. At the hospital, information regarding multiple births, infant gender, gestational age, birth weight, birth length, birth head circumference, maternal age, height, weight before pregnancy, parity, and medical history during pregnancy was collected.

2.2. Exposure measurement. Maternal blood was sampled during the third trimester (N = 356) or during hospitalization within a week after delivery (N = 148). Details of the blood sampling have been reported [25]. All samples were stored at -80°C until analysis. The

concentrations of dioxins in the blood were measured using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) equipped with a solvent-cut large-volume injection system (SGE Ltd., Victoria, Australia) at the Fukuoka Institute of Health and Environmental Sciences. Details of the dioxin measurements have been reported [29]. The gas chromatograph was an Agilent 6890 (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with an AutoSpecUltima NT (Micromass Ltd., Manchester, UK). Specific congeners of seven PCDDs (2,3,7,8-TetCDD, 1,2,3,7,8-PenCDD, 1,2,3,4,7,8-HexCDD, 1,2,3,6,7,8-HexCDD, 1,2,3,7,8,9-HexCDD, 1,2,3,4,6,7,8-HepCDD, and OctCDD), ten PCDFs (2,3,7,8-TetCDF, 1,2,3,7,8-PenCDF, 2,3,4,7,8-PenCDF, 1,2,3,4,7,8-HexCDF, 1,2,3,6,7,8-HecCDF, 2,3,4,6,7,8-HexCDF, 1,2,3,7,8,9-HexCDF, 1,2,3,4,6,7,8-HepCDF, 1,2,3,4,7,8,9-HepCDF, and OctCDF), four non-*ortho* PCBs (3,4,4',5-TetCB (International Union of Pure and Applied Chemistry (IUPAC) #81), 3,3',4,4'-TetCB (#77), 3,3',4,4',5-PenCB (#126), and 3,3',4,4',5,5'-HexCB (#169)), and eight mono-*ortho* PCBs (2',3,4,4',5-PenCB (#123), 2,3',4,4',5-PenCB (#118), 2,3,4,4',5-PenCB (#114), 2,3,3',4,4'-PenCB (#105), 2,3',4,4',5,5'-HexCB (#167), 2,3,3',4,4',5-HexCB (#156), 2,3,3',4,4',5'-HexCB (#157), and 2,3,3',4,4',5,5'-HepCB (#189)) were analyzed, and total dioxin levels were defined as the sum of the levels of all 29 congeners. Seventy-eight blood samples were not available for dioxin measurement or lacked sufficient blood volume for the dioxin measurement; and we measured the levels of dioxin congeners in 426 samples. Details of the detection limit (DL) for each of congener have been previously reported [8]. Sample values below the DL were assigned a value of one-half the DL to estimate each total level. In addition, lipid adjustments were adopted for the calculation of dioxins, as well. The remaining maternal blood samples in this study were not analyzed because they were not available or lacked sufficient blood volume for the dioxin measurement. The TEQs were calculated by multiplying the levels of congeners by the

corresponding toxic equivalency factor as reported by the World Health Organization 2006 [7].

2.3. Genetic analyses. Maternal blood samples were collected at the time of study enrollment, and genomic DNA was extracted from lymphocytes with standard techniques [30]. Because we had observed an association between prenatal dioxin levels and *AHR* and *CYP1A1* genotypes [14] and between prenatal smoking and maternal *AHR*, *CYP1A1* and *GSTM1* genotypes and reduced birth weight [22, 31], we evaluated those three genetic polymorphisms in the present study. The *AHR* (G>A, rs2066853) and *CYP1A1* (T>C, rs4646903) polymorphisms were determined using PCR [32, 33]. *GSTM1* null and non-null genotypes were determined using multiplex PCR [30, 32]. We determined the three polymorphisms in 496 samples. Eighteen blood samples were not analyzed because of insufficient blood volumes. We categorized the genotypes as GG versus GA/AA for the *AHR* polymorphism, TT/TC versus CC for the *CYP1A1* polymorphism, and non-null (Insert/Insert and Insert/Deletion genotype) versus null (Deletion/Deletion genotype) for the *GSTM1* polymorphism.

2.4. Statistical methods. We obtained a complete set of data on dioxin levels, polymorphisms, self-administered questionnaire, and medical history from 422 participants. However, one sample had an extremely high PCDF level and was excluded from the study [14]. Associations between variables were analyzed with the Spearman's correlation test, Mann-Whitney *U*-test, and Kruskal-Wallis test. The total of 29 congener dioxin TEQ values had a non-normal distribution; therefore, log₁₀-transformed each value. First, a multiple linear regression model was used to evaluate the dioxin TEQ in relation to birth weight, birth length, and birth head circumference stratified by maternal genetic polymorphisms with adjustment

for the following covariates: maternal age (years), height (cm), weight before pregnancy (kg), parity (primiparous or multiparous), caffeine intake (mg/day), alcohol intake (g/day), smoking status during pregnancy (non-smoking or smoking), educational level (≤ 9 , 10–12, 13–16, or ≥ 17 years), annual household income (≤ 3 , 3–5, 5–7, 7–10, or >10 million yen), inshore fish intake during pregnancy, deep-sea fish intake during pregnancy (intake of each fish type: almost every day, 3–4 times/week, 1–2 times/week, 1–2 times/month, or never), blood sampling period (during the third trimester or postpartum), infant gender (male or female), and gestational age (weeks). We chose these confounding factors because maternal age, height, weight before pregnancy, parity, caffeine and alcohol intake, smoking status during pregnancy, educational level, annual household income, infant gender and gestational age have been related to infant birth size [31, 34, 35, 36, 37], and maternal age, parity, smoking status during pregnancy, inshore and deep-sea fish intake, and blood-sampling period have been related to maternal dioxin levels [5, 38, 39, 40, 41]. Next, we examined the association of total dioxin TEQ with infant birth size (birth weight, birth length, and head circumference) stratified by maternal *AHR*, *CYP1A1* and *GSTM1* genotypes using a multiple regression analysis. We designated those participants with dioxin TEQ and expected low-risk genotype *AHR*-GG, *CYP1A1*-CC, and *GSTM1*-non-null. Finally, we used a multiple regression model to examine the association of the TEQ of each of 29 dioxin congener with infant birth weight stratified by maternal *GSTM1* genotypes because we found, in this study, that the associations of total dioxin TEQ with infant birth length and head circumference stratified by maternal *AHR* and *CYP1A1* genotypes were not significant. We tested the effect modification using the genes $\times$ dioxin TEQ interaction terms (e.g. null *GSTM1* genotype $\times$ dioxin TEQ). The interaction *P*-value (P_{int}) was calculated using a post-estimation combined *F*-test for the two interaction variables between genotypes and dioxins. Results were considered significant if $P < 0.05$. Interaction results were considered significant if $P < 0.10$.

For multiple comparison, the alpha level was set at 0.025 (0.05/2) in accordance with the Bonferroni correction.

All statistical analyses were performed using JMP Pro11 (SAS Institute Inc., Cary, NC, USA) and SPSS (SPSS Inc., Chicago, IL, USA) software for Windows version 22.0J.

2.5. Approval This study was conducted with written informed consent from all participants and was approved by the institutional ethical board for human genome and genome studies at the Hokkaido University Graduate School of Medicine.

3. Results

Table 1 shows the characteristics of the mothers and infants. Infant birth size was significantly associated with gender and gestational age.

In pregnant women with the *GSTM1* null genotype, we observed a 345-g reduction in infant birth weight for each 10-fold increase in their total dioxin TEQ (95% confidence interval (CI): -584, -105) (Table 2). However, P_{int} for the interaction between total dioxin and *GSTM1* null genotype was not significant (Table 2).

Next, we examined the association between the TEQ for each of the 29 dioxin congeners and infant birth weight stratified by *GSTM1* genotype: three out of 29 congeners were significantly associated. Among the pregnant women with the *GSTM1* null genotype, a 214-g reduction in infant birth weight was found for each 10-fold increase in 2,3,7,8-TetCDD TEQ (95% CI: -413, -16; $P_{\text{int}} = 0.063$); a 359-g reduction for each 10-fold increase in 1,2,3,7,8-PenCDD TEQ (95% CI: -569, -148; $P_{\text{int}} = 0.067$); and a 346-g reduction for each 10-fold increase in 2,3,4,7,8-PenCDF TEQ (95% CI: -567, -126; $P_{\text{int}} = 0.031$; Table 3).

4. Discussion

Our previous study showed that prenatal exposure to dioxins is associated with reduced infant birth size [13]. To the best of our knowledge, this is the first study to identify an association between infant birth size and total dioxin and 2,3,7,8-TetCDD, 1,2,3,7,8-PenCDD, and 2,3,4,7,8-PenCDF TEQs in mothers with the *GSTM1* null genotype. Dioxin levels in the *GSTM1* null genotypes were not significantly different than in those with the *GSTM1* non-null genotype (data not shown). Moreover, the maternal *GSTM1* non-null and null genotypes were not significantly associated with infant birth size (data not shown). Therefore, we considered that infant birth size reduction might be affected by a combination of dioxin levels and maternal *GSTM1* null genotype rather than dioxin level alone.

The *AHR*-GA/AA genotype (compared with the GG genotype), *CYP1A1*-TC/CC genotype (compared with the TT genotype), and *GSTM1* null genotype (compared with the non-null genotype) result in decreased expression of their respective proteins [21, 22, 42, 43]. In our previous study, pregnant women with the *AHR*-GA/AA genotype had higher dioxin levels than those with the *AHR*-GG genotype, and similarly, pregnant women with the *CYP1A1*-TT/TC genotype showed higher dioxin levels than those harboring *CYP1A1*-CC genotype [14]. Pregnant women with the *AHR*-GA/AA, or those with *CYP1A1*-TT/TC genotypes may have compromised dioxin metabolic function and thus, may store residual dioxins. In the previous study, we found that PCDD and PCDF levels are associated with maternal *CYP1A1* polymorphisms, while dioxin-like PCB levels are associated with maternal *AHR* polymorphisms [14]. Dioxin-like PCBs may primarily bind AHR and be metabolized by *CYP1A1* and *GSTM1*. Therefore, we suggest that the dioxin-like PCB levels in conjunction with all the genotypes except *CYP1A1*-TT/TC and *GSTM1* null genotypes were not be associated with birth size reduction.

We observed a nearly significant interaction between the TEQs of three dioxin

congeners and *GSTM1* null genotype, indicating gene-environment interaction. Similarly, Danileviciute et al. [44] demonstrated that prenatal smoking interacted with the *GSTM1* null genotype to reduce infant birth weight. Considering these findings, the high proportion of smokers during pregnancy in our study population (17.1% versus 8.5% in the general Japanese population [45]) should be taken into account when interpreting the results [13, 14, 22]. However, despite the limited sample size ($N = 421$) was relatively small, we detected a small gene-environment effect by analyzing the specific congener analyses.

The exact mechanisms underlying the reported interaction remains unknown, as dioxin TEQ levels in women with different *GSTM1* genotypes do not differ (data not shown). We speculate that the different sets of specific dioxins that can be metabolized by *GSTM1* might explain the relationship between *GSTM1* null genotype and the levels of the three dioxin congeners on birth size. Polycyclic aromatic hydrocarbons (PAHs) are structurally similar to dioxins. Lymphocyte PAH-DNA adduct levels in healthy bus drivers in urban areas have been related to their exposure to air pollution and the null *GSTM1* genotype [46]. Therefore, the presence of unmetabolized dioxins might, therefore, lead to DNA adducts in pregnant women with the *GSTM1* null genotype. However, this hypothesis needs further testing. We would like to assess how gene-environment interactions affect infant development; however, this requires a large study cohort.

The strengths of the current study are the prospective birth cohort design, which minimizes recall bias, and the fact that prenatal dioxin levels are accurate because of the high-sensitivity method of HRGC/HRMS [29]. However, this study also had some limitations. We applied a multiple comparison test to investigate the association among prenatal dioxins, maternal genotypes, and birth outcomes. When we assumed to examine a few hundreds of statistical tests, significant P values were disappeared. However, when we only examined the association of total dioxin TEQ with infant birth weight, P -values remained significant.

Therefore, we needed to pay attention to how we interpreted our data. By doing so, we were able to identify the detrimental gene-environment effects of prenatal exposure to dioxins in mothers having the *GSTM1* null genotype on birth weight.

Conclusion

Infants of mothers with the ~~null~~ *GSTM1* null genotype had lower birth sizes than infants born to mothers with the *GSTM1* non-null genotypes. Chemical exposure during pregnancy and birth size reduction are associated with an increased risks for several disorders such as infant and adult neurodevelopment delays and obesity throughout life based on the developmental origins of health and disease (DOHaD) [47, 48, 49, 50, 51]. The relationship between maternal high-risk genetic groups and maternal dioxin levels with infant birth size should be recognized. Hence, public health approaches should assess the risk of environmental chemical internalization in high-risk genetic groups and provide preventive protocols.

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Conflicts of interest

The authors have no conflicts of interest relevant to this article to disclose.

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Table 1. Infant birth size in relation to maternal and infant characteristics (N = 421)

Characteristic	N (%)	Birth weight (g)		Birth length (cm)		Birth head circumference (cm)	
		Mean (SD)	P value	Mean (SD)	P value	Mean (SD)	P value
Infants							
Gender ^a							
Male	201 (47.7)	3117 (393)	0.007**	48.4 (2.1)	0.002**	33.7 (1.3)	<0.001***
Female	220 (52.3)	3017 (366)		47.8 (1.7)		32.9 (1.2)	
Gestational age (weeks) ^c	38.9 (1.4) ^d	$\rho = 0.416^f$	<0.001***	$\rho = 0.393^f$	<0.001***	$\rho = 0.153^f$	0.002**
Birth weight (g) ^c	3065 (382) ^d			$\rho = 0.701^f$	<0.001***	$\rho = 0.502^f$	<0.001***
Birth length (cm) ^c	48.1 (2.0) ^d	$\rho = 0.701^f$	<0.001***			$\rho = 0.390^f$	<0.001***
Birth head circumference (cm) ^c	33.3 (1.3) ^d	$\rho = 0.502^f$	<0.001***	$\rho = 0.390^f$	<0.001***		
Mothers							
Age (years) ^c	30.8 (4.7) ^d	$\rho = -0.037^f$	0.455	$\rho = -0.058^f$	0.233	$\rho = -0.001^f$	0.986
Height (cm) ^c	158 (5) ^d	$\rho = 0.108^f$	0.027*	$\rho = 0.118^f$	0.015*	$\rho = 0.124^f$	0.011*
Weight before pregnancy (kg) ^c	53 (9) ^d	$\rho = 0.156^f$	0.001**	$\rho = 0.140^f$	0.004**	$\rho = 0.105^f$	0.031*
Parity ^a							
Primiparous	204 (48.5)	3045 (380)	0.309	48.1 (2.0)	0.865	33.2 (1.3)	0.108
Multiparous	217 (51.5)	3083 (384)		48.1 (1.9)		33.4 (1.3)	
Smoking status during pregnancy ^a							
Non-smoking	349 (82.9)	3070 (394)	0.537	48.1 (2.0)	0.409	33.3 (1.4)	0.251
Smoking	72 (17.1)	3040 (322)		47.9 (1.6)		33.1 (1.2)	
Alcohol intake during pregnancy ^a							
No	293 (69.6)	3047 (399)	0.148	48.0 (2.1)	0.123	33.2 (1.4)	0.273
Yes	128 (30.4)	3106 (339)		48.3 (1.7)		33.4 (1.3)	
Alcohol consumption by drinkers (g/day) ^c	1.2 (0.3–51.8) ^e	$\rho = -0.003^f$	0.976	$\rho = -0.071^f$	0.427	$\rho = -0.090^f$	0.315
Caffeine intake during pregnancy (mg/day) ^c	117.3 (1.5–646.3) ^e	$\rho = -0.075^f$	0.125	$\rho = -0.053^f$	0.279	$\rho = 0.005^f$	0.918
Fish intake during pregnancy							
Inshore fish ^b							
Never	20 (4.8)	3104 (417)	0.322	47.6 (2.1)	0.571	33.3 (1.0)	0.309
1–2 times/month	210 (49.9)	3085 (347)		48.2 (1.7)		33.4 (1.3)	
1–2 times/week	167 (39.7)	3028 (423)		48.0 (2.2)		33.1 (1.4)	
3–4 times/week	23 (5.5)	3139 (329)		48.3 (1.5)		33.5 (1.3)	
Almost every day	1 (0.2)	2604 (0)		47.1 (0)		32.5 (0)	
Deep-sea fish ^b							
Never	12 (2.9)	3096 (508)	0.996	47.8 (2.5)	0.975	33.3 (0.9)	0.350
1–2 times/month	182 (43.2)	3066 (380)		48.0 (1.8)		33.4 (1.3)	
1–2 times/week	201 (47.7)	3060 (393)		48.1 (2.1)		33.2 (1.4)	
3–4 times/week	25 (5.9)	3083 (251)		48.1 (1.6)		33.5 (1.2)	
Almost every day	1 (0.2)	3098 (0)		47.5 (0)		33.0 (0)	
Educational level (years) ^b							
≤9	9 (2.1)	3209 (417)	0.446	48.7 (1.8)	0.131	33.1 (1.8)	0.791
10–12	168 (39.9)	3040 (390)		47.9 (1.9)		33.2 (1.4)	

13–16	235 (23.5)	3073 (380)		48.1 (2.0)		33.3 (1.3)	
≥17	9 (2.1)	3161 (233)		49.3 (1.3)		33.3 (0.5)	
Annual household income (million yen) ^b							
≤3	68 (16.2)	3071 (404)	0.073	47.9 (2.1)	0.237	33.1 (1.4)	0.138
3–5	209 (49.6)	3083 (367)		48.1 (2.0)		33.4 (1.3)	
5–7	93 (22.1)	3008 (382)		48.1 (1.8)		33.1 (1.3)	
7–10	44 (10.5)	3034 (385)		47.8 (2.1)		33.4 (1.5)	
≥10	7 (1.7)	3409 (474)		49.5 (0.9)		34.0 (0.8)	
Blood sampling period ^a							
During pregnancy	293 (69.6)	3072 (389)	0.568	48.2 (2.0)	0.193	33.3 (1.3)	0.821
Postpartum	128 (30.4)	3049 (366)		47.9 (1.9)		33.3 (1.4)	
AHR (G>A, Arg554Lys) ^b							
GG	142 (33.7)	3049 (414)	0.793	48.1 (1.8)	0.998	33.3 (1.4)	0.897
GA	195 (46.6)	3078 (364)		48.1 (2.1)		33.3 (1.3)	
AA	84 (20.0)	3061 (370)		48.1 (1.9)		33.3 (1.3)	
CYP1A1 (T6235C) ^b							
TT	176 (41.8)	3086 (339)	0.116	48.2 (1.8)	0.344	33.3 (1.3)	0.681
TC	201 (47.7)	3029 (404)		47.9 (2.1)		33.3 (1.3)	
CC	44 (10.5)	3145 (430)		48.2 (1.7)		33.3 (1.3)	
GSTM1 (Insert/Deletion) ^a							
Non-null	209 (49.6)	3062 (372)	0.876	48.1 (1.8)	0.453	33.3 (1.4)	0.631
Null	212 (50.4)	3068 (392)		48.0 (2.1)		33.3 (1.3)	

^a Mann-Whitney *U*-test. ^b Kruskal-Wallis test. ^c Spearman's correlation test.

^d Mean (SD). ^e Median (Minimum–Maximum). ^f Spearman's correlation (ρ).

*, **, *** Statistically significant differences ($P < 0.05$, $P < 0.01$, $P < 0.001$, respectively).

Table 2. Regression coefficients (95% CIs) of total dioxins (pg TEQ/g lipid) during pregnancy and maternal *AHR*, *CYP1A1*, and *GSTM1* genotypes for infant birth weight, birth length, and birth head circumference

Combined genotypes	Birth weight (g) ^a	Birth length (cm) ^a	Birth head circumference (cm) ^a
<i>AHR</i>			
GG	-184 (-469, 102)	-0.10 (-1.64, 1.44)	0.28 (-0.83, 1.40)
GA/AA	-214 (-438, 10)	-0.03 (-1.24, 1.18)	-0.46 (-1.33, 0.41)
	$P_{int} = 0.797$	$P_{int} = 0.993$	$P_{int} = 0.258$
<i>CYP1A1</i>			
CC	-146 (-356, 64)	-0.10 (-1.24, 1.04)	-0.24 (-1.07, 0.58)
TT/TC	-202 (-387, -17)*	-0.12 (-1.12, 0.88)	-0.21 (-0.93, 0.51)
	$P_{int} = 0.980$	$P_{int} = 0.935$	$P_{int} = 0.300$
<i>GSTM1</i>			
Non-null	-69 (-321, 182)	0.37 (-0.98, 1.73)	-0.01 (-1.00, 0.97)
Null	-345 (-584, -105)**	-0.72 (-2.01, 0.57)	-0.51 (-1.44, 0.43)
	$P_{int} = 0.118$	$P_{int} = 0.233$	$P_{int} = 0.474$

Total dioxins were defined as the sum of 29 congeners.

AHR, G>A, Arg554Lys; *CYP1A1*, T6235C; *GSTM1*, non-null/null, Insert/Deletion.

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

^a Because dioxin TEQs were log₁₀-transformed, partial regression coefficients represent the expected change in dependent variables as a result of a 10-fold change in dioxin TEQ.

The interaction P -value (P_{int}) was calculated using a post-estimation combined F -test for the two interaction variables between genotype and total dioxin TEQ (e.g., *AHR* GG × total dioxin TEQ).

*, ** Statistically significant, P -value < 0.05 and 0.01, respectively.

Table 3. Regression coefficients (95% CI) of maternal 2,3,7,8-TetCDD, 1,2,3,7,8-PenCDD, and 2,3,4,7,8-PenCDF (pg TEQ/g lipid) during pregnancy and maternal *GSTM1* genotypes for infant birth weight

	<i>GSTM1</i> genotypes		P_{int}
	Non-null	Null	
PCDDs			
2,3,7,8-TetCDD	38 (-149, 224)	-214 (-413, -16)*	0.063
1,2,3,7,8-PenCDD	-91 (-314, 132)	-359 (-569, -148)**	0.067
PCDFs			
2,3,4,7,8-PenCDF	-35 (-241, 172)	-346 (-567, -126)**	0.031

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

Because dioxin congener TEQs were log₁₀-transformed, partial regression coefficients (change of birth weight (gram)) represent the expected change in dependent variables as a result of a 10-fold change in dioxin congener TEQ.

Interaction P -value (P_{int}) was calculated using a post-estimation combined F -test for the two interaction variables between genotype and each dioxin congener TEQ (e.g., null *GSTM1* genotype $\times$ 2,3,7,8-TetCDD TEQ).

*, **, *** Statistically significant, P -value <0.05, 0.01 and 0.001, respectively.