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Title

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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Abbreviations

PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; PCBs, polychlorinated biphenyls; 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 ; TEF, toxicity equivalence factor; SNPs, single-nucleotide polymorphisms; LOD, limit of detection; PenCB, pentachlorinated biphenyl; ADP, adenosine diphosphate; E_2, 17ß-estradiol; E_1, estrone; 2-OH-E_2, 2-hydroxyestradiol; 4-OH-E_2, 4-hydroxyestradiol; ERα, estrogen receptor α; TSH, thyroid-stimulating hormone; TSHß, thyroid-stimulating hormone, β subunit; E_2-ERα, 17ß-estradiol-bound estrogen receptor α; T_3, tri-iodothyronine

Highlights

- We examined the association of dioxin concentrations with genetic susceptibility.
- Six polymorphisms of dioxin-metabolizing enzymes were investigated.
- Six polymorphisms were detected in 421 healthy pregnant Japanese women.
- There were different blood concentrations and TEQ with both *AHR* (rs2066853) and *CYP1A1* (rs4646903).
- Polymorphisms of *AHR* and *CYP1A1* were associated with maternal dioxin concentrations and TEQ.

Abstract

Dioxins are metabolized by cytochrome P450, family 1 (CYP1) via aromatic the hydrocarbon receptor (AHR). Our aim was to determine 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. The differences in exposure concentrations of dioxins in maternal blood among the genotypes were investigated. At first, a comparison among GG, GA and AA of *AHR* showed significant difference (genotype model: P=0.016 for the mono-*ortho* PCBs concentrations and toxicity equivalency quantity (TEQ)). Secondly, there was significant association between the dominant genotype model [(TT+TC) vs. CC: P=0.048 for PCDD TEQ; P=0.035 for PCDF TEQ] of *CYP1A1* (rs4646903). No significant differences were obtained among blood dioxin concentrations and polymorphisms of *AHRR*, *CYP1A1* (rs1048963), *CYP1A2,* and *CYP1B1*. Thus, polymorphisms of *AHR* and *CYP1A1* (rs4646903) were associated with maternal dioxin concentrations. However, differences in dioxin blood concentrations were relatively low.

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 ingesting 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 altered immunologic response and 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 following exposure through circulating blood via the placenta (Miller et al., 2004; Chao et al., 2007). Exposure to high levels of PCDDs plus PCDFs (median blood concentration of 168 pg/g lipid) in pregnant women has been 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 (TEQ) pg/g lipid] 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 exposed to low dioxin levels did not give birth to babies with low or reduced birth weight (Longnecker et al., 2005; Nishijo et al., 2008). These conflicting results suggest that maternal genetic susceptibility in regard to enzymes concerned with dioxin metabolism may be involved.

Dioxins, which include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD),

bind 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-suppression factor AHR repressor (AHRR). Genetic polymorphisms in *AHR*, *AHRR,* and *CYP* modulate the degree of disease risks. 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), and 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, these polymorphisms might affect dioxin blood concentrations. Second, the exposure concentration may affect the reproductive and immune systems. Third, effects on these systems might lead to increased risk for the various diseases.

Exposure to low levels of dioxins might cause reproductive toxicity as described previously (Tajimi et al., 2005; Sonneborn et al., 2008; Konishi et al., 2011). Dioxins share a metabolic pathway via AHR and the CYP1 family enzymes with polycyclic aromatic hydrocarbons, which are a component of cigarette smoke. The risk of fetal growth restriction in pregnant women who smoke during pregnancy is modulated by maternal *CYP1A1*, glutathione *S*-transferase θ1 (*GSTT1*), and glutathione *S*-transferase μ1 (*GSTM1*) polymorphisms (Delpisher et al., 2009). In the

same way, differences in genetic susceptibility to environmental chemicals in the parental generation may cause adverse health effects in the offspring. Maternal genotypes of *GSTM1* null, a *CYP1A1* (rs1048963) variant, and the combination of *GSTM1* null and *CYP1A1* (rs4646903) variant alleles 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, genetic susceptibility in pregnant women to environmental chemicals might affect the health status in the next generation. There are little understanding of the association between environmental exposed levels of chemicals included dioxins and fetal and children's development not only in years later from birth. Dioxin-like polychlorinated biphenyl (PCB) (IUPAC No.126) was around 10,000-fold more potent than non-dioxin-like PCB (IUPAC No.153) during pregnancy exposed, their isomers impaired learning ability in young rats (three months), and affected in males and females similarly (Piedrafita et al., 2008). However, the underlying mechanisms remain unclear in human. From now, we will investigate the effects of dioxins on the developing school-age children. The last we need to examine associations between dioxin concentrations and polymorphisms of dioxin-metabolizing genes and evaluate the gene-environment interactions. Consequently, we examined the association of dioxin concentrations in the blood with genetic susceptibility in healthy mothers. The objective of this study was to determine whether there are differences in exposure concentrations of dioxins among *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, after obtaining informed consent, we recruited pregnant women from Sapporo Toho Hospital in Hokkaido, northern Japan. Details of the cohort study methods have been reported previously (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. Subjects 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 514 registered participants of the cohort study and 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 according to a previously published method (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. Toxicity equivalence quantity (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 on a 7300/7500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA) (Ranade et al., 2001). 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

(LOD) when the levels were below the LOD for individual congeners. Associations between dioxin concentrations and TEQ and genotypes of *AHR* (rs2066853), *AHRR* (rs2292596), *CYP1A1* (rs4646903 and rs1048963), *CYP1A2* (rs762551), and *CYP1B1* (rs1056836) were analyzed with the 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. The associations between *AHR, AHRR, CYP1A1, CYP1A2* and *CYP1B1* polymorphisms and dioxin concentrations and TEQ were assessed by generalized linear model and *P*-values under a genotype model, a dominant model [(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*; (GG+GC) vs. CC for *CYP1B1*] and recessive model [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*; GG vs. (GC+CC) for *CYP1B1*], respectively (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 participated 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 percentage of subjects that drank alcohol and smoked during pregnancy was 30.4% and 17.1%, respectively. The majority of subjects 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* (rs4646903; T>C and rs1048963; A>G), *CYP1A2* (A>C), and *CYP1B1* (C>G) polymorphisms have minor allele frequencies of 43.1%, 39.8%, 34.3%, 22.1%, 37.2%, and 13.4%, respectively, among pregnant Japanese women in this study.

Table 3 and 4 shows that adjusted mean (95% confidence interval) concentrations and TEQs in the generalized linear model (GLM model) of total PCDDs, PCDFs, and dioxin-like PCBs among *AHR* (rs2066853) (Table 3) and *CYP1A1* (rs4646903) polymorphisms (Table 4) of pregnant women in Sapporo, Hokkaido, Japan, and Figure 1 and 2 shows that adjusted mean concentrations (Figure 1) and TEQs (Figure 2) in the GLM model of congeners.

At first, a comparison among GG, GA and AA of *AHR* (rs2066853) showed significant difference (genotype model: P=0.016 for the mono-*ortho* PCBs concentrations and TEQ; P=0.014 for the total dioxin concentrations). In addition, there was also significant association between 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 and TEQ and total dioxin concentrations] (Table 3).

A comparison among TT, TC and CC of *CYP1A1* (rs4646903) showed no significant difference. However, there was significant association between the dominant genotype model [(TT+TC) vs. CC: P=0.048 for PCDD TEQ; P=0.035 for PCDF TEQ, respectively] (Table 4).

Secondly, in a stratified analysis by congener concentrations of 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 [(AA+AG) vs. GG] showed 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) concentration, respectively]. Furthermore, 2,3,4,7,8-PeCDF concentrations of *CYP1A1* (T>C, *MspI*) genotype model and dominant model showed significant difference [genotype model (TT vs. CC) and dominant model (TT+TC vs. CC): P=0.049 and P=0.028, respectively] (Figure 1).

At last, in a stratified analysis by congener TEQs of 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 [(AA+AG) vs. GG] showed 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=0.014 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 *CYP1A1* (T>C, *MspI*) genotype model and dominant model showed significant difference [genotype model (TT vs. CC) and dominant model (TT+TC vs. CC): P=0.045 and P=0.028, respectively] (Figure 2).

In contrast, no significant differences were obtained in dioxin concentrations and TEQ 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 mental and motor development at the age of 6 months (Nakajima et al., 2006) and birth weight (Konishi et al., 2009) 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; Sasaki et al., 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 have the sensitivity of AHR. Although the toxic effects of TCDD have been studied for several decades, its detailed molecular mechanisms are still poorly understood except for the TCDD-mediated transcriptional regulation of AHR and its binding with AHR nuclear translocator (ARNT) (Gim et al., 2010). Accumulating in fatty tissue, TCDD 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 the metabolic activation of hydrophobic molecules such as PCDDs (Ziegler, 1991). CYP1B1 has catalytic activities that 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 17ß-estradiol in human breast carcinoma cells through alteration of CYP1A1 and CYP1B1 expression (Lin et al., 2008). CYP1A1 and CYP1B1

mediate the transformation of 17ß-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). In fact, 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 single strands (Miura et al., 2000; Lin et al., 2003). Cells treated with E_2 + 2-OH-E_2 exhibit a significant decrease in the estrogen-induced response (Gupta et al., 1998).

TCDD mediates estrogen receptor alpha (ERα) signaling in MCF-7 cells under moderate 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, so AHR-mediated inhibition occurs by estradiol-induced transactivation. TCDD induces interaction of AHR with ERα in the presence of estradiol (Wormke et al., 2003).

E_2-ERα inhibits thyroid-stimulating hormone, β subunit (*TSHß*) expression (Nagayama et al., 2008). Transcriptional repression of *TSHß* is regarded to be specific to triiodothyronine (T_3) and its receptor. The proinflammatory cytokine interleukin-1ß 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). It is possible that effects caused by a lack of thyroid hormones in pregnant women with poor dioxin-metabolizing enzyme activity might impair fetal brain development and also contribute to hypothyroidism in the fetus.

To the best of our knowledge, this is the first study to show that there

are different dioxin blood levels among subjects 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 *AHR*, *ARNT*, and *CYP1B1* mRNA expression (Helmig et al., 2011). It has thus been suggested that *AHR* AA might reduce AHR activity and decrease metabolism by CYP1.

CYP1A1 activity is significantly higher among persons with the *CYP1A1* (rs4646903) TC or CC genotype (Landi et al., 1994). It has been suggested that dioxin levels might be influenced by CYP1A1 activity or CYP1A1 expression.

In our previous study, (1) -231.5 g and -258.8 g changed in birth weight for a 10-fold increase in the TEQ levels of total PCDDs and PCDFs, respectively (Konishi et al., 2009). (2) Total PCDD concentrations were significantly negative associated with Bayley scales of infant development (BSID)-II mental development index (MDI) score in six months (β=-0.234 was the point increase in development score per total PCDD level [natural logarithm] (Nakajima et al., 2006). (3) 2.50 in odds ratio of otitis media for 75-100th percentiles TEQ (3.06-7.77 TEQ pg/g lipid) of total PCDFs increased compared to 0-25 percentiles TEQ (0.64-1.79 TEQ pg/g lipid) (Miyashita et al., 2011). (4) -211 g and -1.2 cm changed in birth weight and length for infants born to smoking women having *AHR* (G>A, Arg554Lys) GG compared to those born to non-smoking women having *AHR* GA+AA, -170 g and -0.8 cm changed in birth weight and length for infants born to smoking women having *CYP1A1* (T>C, *MspI*) TC+CC compared to those born to non-smoking women having *CYP1A1* TT, -315 g and -1.7 cm changed for infants born to smoking women having *AHR* GG, *CYP1A1* TC+CC compared to those born to non-smoking women having *AHR* GA+AA, *CYP1A1* TT, respectively

(Sasaki et al., 2006). In 82 children aged 6-10 years attending schools near the industrial area in Mexico, Sanchez-Guerra et al. (2012) investigated association between *CYP1A1*2C, CYP1B1*3, GSTM1*0 and GSTT1*0* polymorphism, urinary 1-hydroxypyrene (1-OHP; a biomarker of polycyclic aromatic hydrocarbons exposure) and DNA adducts, and observed higher urinary 1-OHP concentration and for *CYP1A1*2C* AG+GG only compared to *CYP1A1*2C* AA (0.23 μmol/mol creatinine for AA vs. 0.45 μmol/mol creatinine for AG+GG). In human term placental trophoblast cultures after prototype AHR ligands/activators (2,3,7,8-TCDD and 3-methylcholanthlene), CYP1A1 messenger RNA (mRNA), but not CYP1A2, CYP1B1, AHR and AHRR mRNA, was significantly induced (Stejskalova et al., 2011). In present study, dioxin-like PCB concentrations and TEQ were significantly decreased *AHR* (G>A, Arg554Lys) GA+AA compared to GG and, PCDFs was significantly decreased *CYP1A1* (T>C, *MspI*) TC+CC compared to TT, too. After adjusted smoking status during pregnancy, changes of dioxin concentrations and TEQ were significantly decreased for *AHR* and *CYP1A1*, not *AHRR, CYP1A2* and *CYP1B1*. Compared to previous study of Sasaki et al. (2006), Sanchez-Guerra et al. (2012) and Stejskalova et al. (2011), we observed only statistically significant in the *AHR* and *CYP1A1,* but not statistically significant in *AHRR, CYP1A2* and *CYP1B1* as well as previous three reports. Furthermore, chemical effects among tobacco smoke including polycyclic aromatic hydrocarbon might be larger confounding factor of *AHR* and *CYP1A1* genotypes for pregnant women exposed to dioxins of low levels. However, associations between *AHR* (G>A, Arg554Lys) or *CYP1A1* (T>C, *MspI*) genotype and dioxin concentrations in human are still unclear now. In our study, we observed about 1.1 times differences of dioxin TEQs and concentrations by genotypes. Compared to our previous study of Konishi et al. (2009), about -20 to -25g change (maximum levels) will predict in birth weight for a

1.1-fold increase in the levels of dioxins.

For Japanese pregnant women, 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'-hexachlorinated biphenyl (HexCB) (IUPAC No. 167) showed significant for the *AHR* genotypes. Metabolize 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'-hexachlorinated biphenyl (HexCB) (IUPAC No. 167) were still unclear in human but known just a little in mice. There was typically one dose-response relationship for induction of CYP1A1 and CYP1A2 enzyme activity in which the relative potency was different 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 was altered, but the liver weight was significantly increased by 2,2',4,4',5,5'-HexCB treatment in pregnant mice (Mattsson et al., 1981).

2,3,4,7,8-PeCDF showed significant for the *CYP1A1* (rs4646903) genotypes. Pharmacokinetics of 2,3,4,7,8-PeCDF was known just a little in human. In Yucheng patients in Taiwan who were exposed to high levels of 2,3,4,7,8-PeCDF was described as the most contributor to the toxic effects because this congener 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 control subjects, especially in relation to the respective exposure routes. They referred that between absorptivity and rate of metabolism and elimination for dioxin congeners depended on the exposure source. Further work is need to confirm these finding of *AHR* and *CYP1A1* in dioxin congener's studies by human especially pregnant women, and exposed 2,3',4,4',5-PenCB (IUPAC No. 118), 2,3,3',4,4'-PenCB (IUPAC No. 105), 2,3',4,4',5,5'-hexachlorinated biphenyl (HexCB) (IUPAC No. 167) and 2,3,4,7,8-PeCDF chronically in

environmental lower levels could be causally confirmed by epidemiological study.

Although genetic polymorphisms cannot be changed, it is feasible that adverse health effects of dioxins could be prevented by modulating exposure levels, especially among individuals with increased genetic susceptibility, because dioxins might be a modifiable pollutant in the environment. For example, one way to reduce dioxin exposure in 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 could not measure any metabolites of dioxins or placental AHR and CYP1A1 activity. Because some metabolites were produced from one of the dioxin congeners and it is difficult to distinguish the metabolites from the congeners, 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 lacking exon 8 with the Pro185Ala polymorphism is capable of repressing AHR (Karchner et al., 2009). Further studies are needed to examine whether this mutation has any functional consequences.

In the present study, differences in dioxin blood concentrations were relatively low. However, despite this, differences in the health effects might partially exist depending on 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 the developing school-age children. Now, we are 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, allergies, and growth. If the

results finally come in, we will release a report. And, we will focus our attention not only on the dioxin-metabolizing genes but also on the effects of polymorphisms on sex hormone production. Molecular and genetic epidemiological studies are also needed to further elucidate the effects of both environmental and genetic factors in humans now and in future generations.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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

Characteristic	Value (n = 421)
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 in 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%)

Data are shown as either n (%), mean ± standard deviation, or median (range).

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, *Msp*I, 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)

Table 3. Adjusted mean (95% confidence interval) in the generalized linear model of total PCDDs, PCDFs, and dioxin-like PCBs among *AHR* polymorphisms of pregnant women in Sapporo, Hokkaido, Japan

	AHR (G>A, Arg554Lys, dbSNP ID: rs2066853)					
	GG	GA	AA	GA+AA	GG+GA	*P* value
	Adjusted mean (95%CI)	Adjusted mean (95%CI)	Adjusted mean (95%CI)	Adjusted mean (95%CI)	Adjusted mean (95%CI)	
Concentrations (pg/g lipid)						
PCDDs						
Genotype#	478.5 (444.1-512.9)	519.7 (490.5-548.9)	526.3 (481.6-570.9)			0.097
Dominant##	478.5 (444.1-512.9)			521.7 (497.3-546.0)		0.047*
Recessive###			526.3 (481.6-570.9)		502.4 (480.3-524.5)	0.355
PCDFs						
Genotype#	19.2 (17.3-21.1)	21.0 (19.4-22.7)	20.2 (17.7-22.7)			0.365
Dominant##	19.2 (17.3-21.1)			20.8 (19.4-22.1)		0.189
Recessive###			20.2 (17.7-22.7)		20.3 (19.0-21.5)	0.968
Non-*ortho* PCBs						
Genotype#	74.6 (67.7-81.5)	83.4 (77.6-89.3)	86.1 (77.2-95.1)			0.079
Dominant##	74.6 (67.7-81.5)			84.2 (79.4-89.1)		0.028*
Recessive###			86.1 (77.2-95.1)		79.7 (75.3-84.2)	0.216
Mono-*ortho* PCBs						
Genotype#	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##	11,266.3 (10,265.9-12,266.8)			13,087.0 (12,379.6-13,794.4)		0.004*
Recessive###			12,948.9 (11,650.1-14,247.7)		12,356.1 (11,709.3-13,003.3)	0.434
Total dioxins						

Genotype#	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##	11,838.7 (10,820.5-12,856.9)			13,713.7 (12,993.7-14,433.7)		0.004*
Recessive###			13581.5 (12,259.1-14,904.3)		12,958.9 (12,300.1-13,617.0)	0.419
TEQ						
(TEQ pg/g lipid)						
PCDDs						
Genotype#	7.003 (6.513-7.493)	7.465 (7.050-7.881)	7.472 (6.837-8.108)			0.323
Dominant##	7.003 (6.513-7.493)			7.467 (7.121-7.814)		0.132
Recessive###			7.472 (6.837-8.108)		7.271 (6.957-7.585)	0.583
PCDFs						
Genotype#	2.505 (2.342-2.668)	2.598 (2.460-2.736)	2.571 (2.359-2.782)			0.696
Dominant##	2.505 (2.342-2.668)			2.590 (2.475-2.705)		0.410
Recessive###			2.571 (2.359-2.782)		2.559 (2.455-2.664)	0.927
Non-*ortho* PCBs						
Genotype#	4.179 (3.769-4.590)	4.809 (4.460-5.157)	4.693 (4.160-5.226)			0.068
Dominant##	4.179 (3.769-4.590)			4.774 (4.484-5.064)		0.022*
Recessive###			4.693 (4.160-5.226)		4.544 (4.280-4.809)	0.633
Mono-*ortho* PCBs						
Genotype#	0.338 (0.308-0.368)	0.394 (0.369-0.420)	0.388 (0.350-0.427)			0.016*
Dominant##	0.338 (0.308-0.368)			0.393 (0.371-0.414)		0.004*
Recessive###			0.388 (0.350-0.427)		0.371 (0.351-0.390)	0.434
Total dioxins						
Genotype#	14.025 (13.056-14.995)	15.267 (14.443-16.090)	15.124 (13.865-16.383)			0.145
Dominant##	14.025 (13.056-14.995)			15.224 (14.538-15.910)		0.050

Recessive###	15.124 (13.865-16.383)	14.745 (14.121-15.369)	0.604

95%CI; 95% confidence interval, *; $P < 0.05$.

#; Genotype model, ##; Dominant genotype model, ###; Recessive genotype 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 in the generalized linear model.

Table 4. Adjusted mean (95% confidence interval) in the generalized linear model of total PCDDs, PCDFs, and dioxin-like PCBs among *CYP1A1* polymorphisms of pregnant women in Sapporo, Hokkaido, Japan

	CYP1A1 (T>C, *MspI*, dbSNP ID: rs4646903)					
	TT	TC	CC	TC+CC	TT+TC	*P*value
	Adjusted mean (95%CI)	Adjusted mean (95%CI)	Adjusted mean (95%CI)	Adjusted mean (95%CI)	Adjusted mean (95%CI)	
Concentrations						
(pg/g lipid)						
PCDDs						
Genotype#	529.8 (499.3-560.3)	497.2 (468.6-525.7)	461.9 (400.6-523.1)			0.097
Dominant##			461.9 (400.6-523.1)		512.4 (491.6-533.2)	0.127
Recessive###	529.8 (499.3-560.3)			490.9 (465.0-516.7)		0.057
PCDFs						
Genotype#	20.8 (19.1-22.5)	20.4 (18.8-21.9)	17.9 (14.4-21.3)			0.324
Dominant##			17.9 (14.4-21.3)		20.5 (19.4-21.7)	0.144
Recessive###	20.8 (19.1-22.5)			19.9 (18.5-21.3)		0.454
Non-*ortho* PCBs						
Genotype#	85.5 (79.4-91.6)	78.6 (72.9-84.3)	74.0 (61.6-86.3)			0.139
Dominant##			74.0 (61.6-86.3)		81.8 (77.6-86.0)	0.240
Recessive###	85.5 (79.4-91.6)			77.8 (72.6-83.0)		0.061
Mono-*ortho* PCBs						
Genotype#	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##			11,911.9 (10,112.4-13,711.4)		12,538.3 (11,928.2-13,148.3)	0.518
Recessive###	12,748.0 (11,851.9-13,644.0)			12,275.6 (11,517.7-13,033.5)		0.431
Total dioxins						

Genotype#	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##			12,465.6 (10,634.0-14,297.2)		13,153.0 (12,532.0-13,774.1)	0.486
Recessive###	13,384.0 (12,472.0-14,296.1)			12,864.1 (12,092.7-13,635.6)		0.394
TEQ						
(TEQ pg/g lipid)						
PCDDs						
Genotype#	7.616 (7.183-8.049)	7.225 (6.821-7.630)	6.480 (5.611-7.349)			0.062
Dominant##			6.480 (5.611-7.349)		7.408 (7.113-7.703)	0.048*
Recessive###	7.616 (7.183-8.049)			7.092 (7.182-8.049)		0.072
PCDFs						
Genotype#	2.653 (2.510-2.797)	2.545 (2.411-2.680)	2.267 (1.978-2.555)			0.061
Dominant##			2.267 (1.978-2.555)		2.596 (2.498-2.694)	0.035*
Recessive###	2.653 (2.510-2.797)			2.495 (2.373-2.617)		0.103
Non-*ortho* PCBs						
Genotype#	4.730 (4.363-5.096)	4.496 (4.154-4.839)	4.300 (3.564-5.035)			0.490
Dominant##			4.300 (3.564-5.035)		4.605 (4.356-4.855)	0.441
Recessive###	4.730 (4.363-5.096)			4.461 (4.152-4.771)		0.273
Mono-*ortho* PCBs						
Genotype#	0.382 (0.356-0.409)	0.371 (0.346-0.396)	0.357 (0.303-0.411)			0.666
Dominant##			0.357 (0.303-0.411)		0.376 (0.358-0.394)	0.518
Recessive###	0.382 (0.356-0.409)			0.368 (0.346-0.391)		0.431
Total dioxins						
Genotype#	15.381 (14.521-16.242)	14.638 (13.833-15.442)	13.403 (11.676-15.131)			0.111
Dominant##			13.403 (11.676-15.131)		14.985 (14.398-15.571)	0.090

Recessive###	15.381 (14.521-16.242)	14.417 (13.688-15.146)	0.095

95%CI; 95% confidence interval, *; $P < 0.05$.

#; Genotype model, ##; Dominant genotype model, ###; Recessive genotype 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 in the generalized linear model.

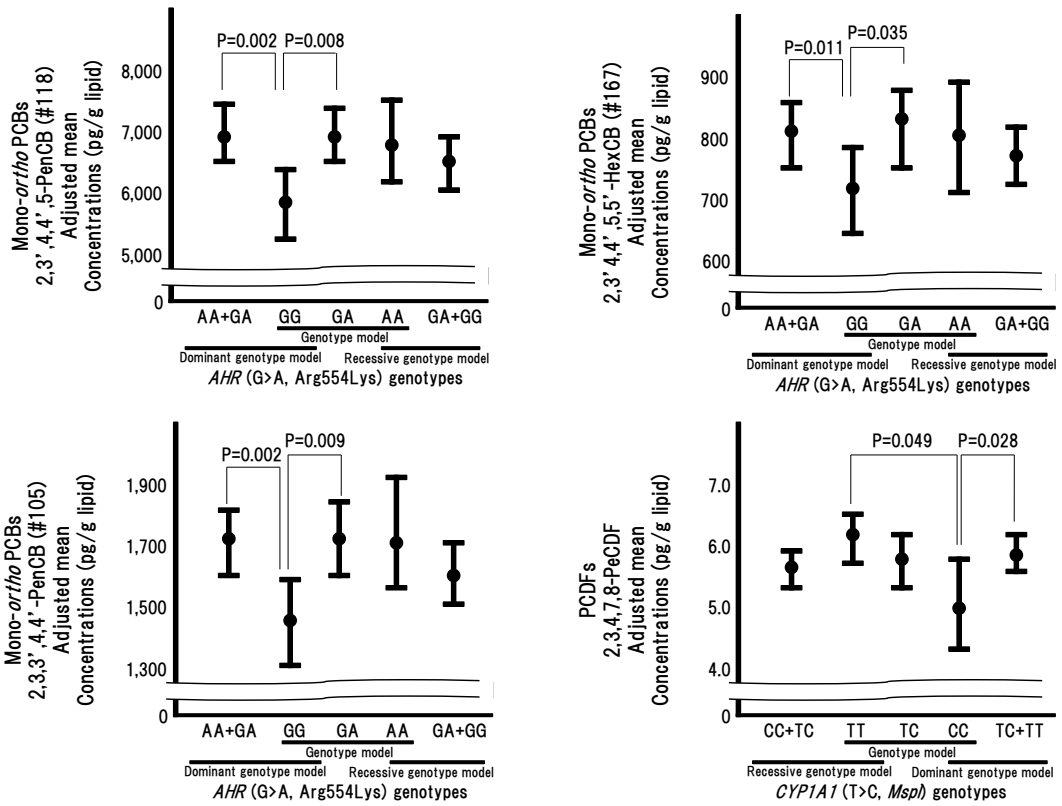


Figure 1. Adjusted mean (95% confidence interval) concentrations in the generalized linear model of dioxin congeners among *AHR* and *CYP1A1* polymorphisms of pregnant women in Sapporo, Hokkaido, Japan

Dot and bar are shown as adjusted mean and 95% confidence interval, respectively.

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.

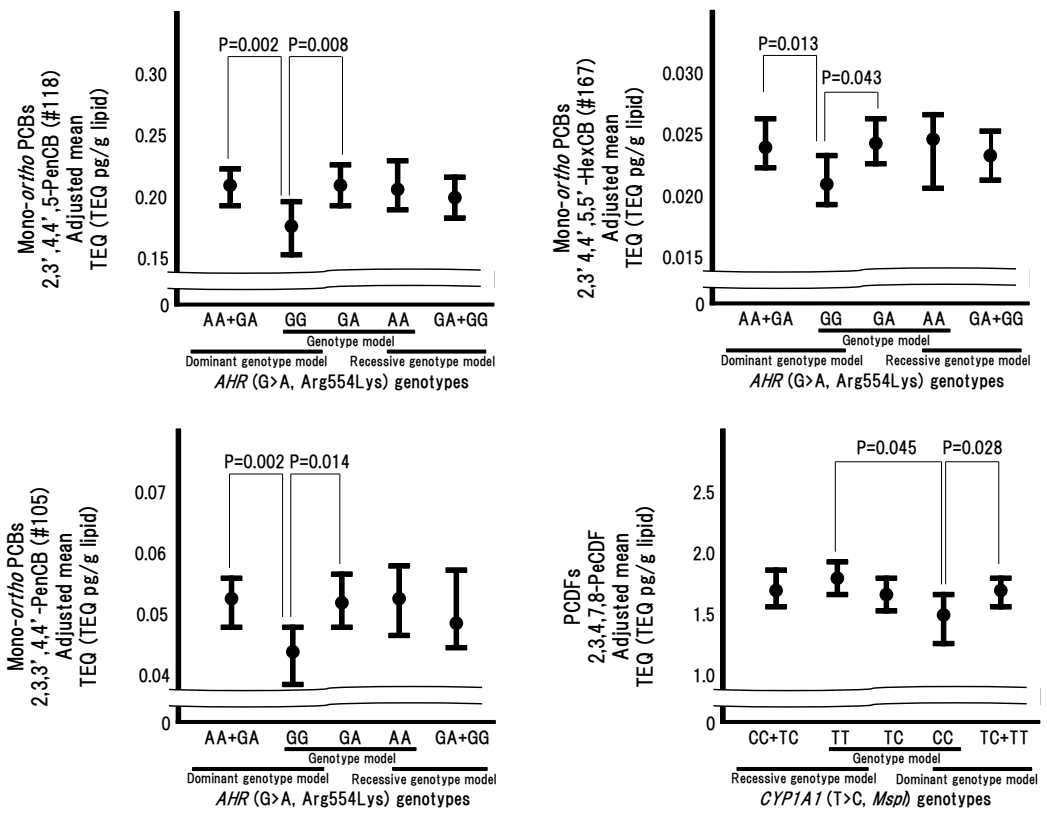


Figure 2. Adjusted mean (95% confidence interval) TEQ in the generalized linear model of dioxin congeners among *AHR* and *CYP1A1* polymorphisms of pregnant women in Sapporo, Hokkaido, Japan

Dot and bar are shown as adjusted mean and 95% confidence interval, respectively.

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.

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