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

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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.

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 [1–4]. 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-tetrachlorinated dibenzo-p-dioxin (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 propor-

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.

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tion 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 [15–17]. 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,