City. The levels of 9 key sex hormones in the cord blood (e.g., Estradiol, Testosterone, Progesterone, etc.) and the levels of 11 fatty acids in the maternal plasma were also measured by liquid chromatography-tandem mass spectrometry (LC–MS/MS) and gas chromatography-mass spectrometry (GC–MS), respectively. In the Hokkaido cohort, maternal serum was used to measure folic acid levels [32].

Exposure measurements

PCBs, OH-PCBs and Dioxins

In the Sapporo cohort, the levels of 29 congeners of dioxins and dioxin-like polychlorinated biphenyls (DL-PCBs) [7 polychlorinated dibenzodioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs), 4 Non-*ortho* PCBs and 8 Mono-*ortho* PCBs], 58 congeners of the other PCBs and 5 congeners of hydroxylated polychlorinated biphenyls (OH-PCBs) in maternal blood and breast milk were measured using a high-resolution gas chromatography/high-resolution mass spectrometer (HRGC/HRMS) at the Fukuoka Institute of Health and Environmental Sciences [33–37]. The Toxicity Equivalency Quantity (TEQ) levels were calculated by multiplying the levels of individual congeners by its toxic equivalency factor (TEF) values of WHO 2005 [38].

PFCs

In the Sapporo cohort, PFOS and PFOA levels in maternal blood, cord blood and breast milk were analyzed by LC–MS/MS at Hoshi University [39, 40]. For the Hokkaido cohort study, among PFCs, 11 perfluoroalkyl acids (PFAAs) [perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexane acid (PFHxS) and perfluorooctane sulfonate (PFOS)] were measured in maternal plasma using simultaneous analysis with ultraperformance liquid chromatography in combination with triple quadrupole mass spectrometry (UPLC-MS/MS) at the Research Faculty of Agriculture, Hokkaido University [41].

Organochlorine pesticides

In the Sapporo cohort, the levels of persistent organochlorine pesticides in maternal blood were analyzed by a gas chromatography/negative-ion chemical-ionization mass spectrometry (GC/NCIMS) and a gas chromatography/high-resolution mass spectrometry (GC/HRMS) at IDEA Consultants, Inc. [42].

Metals

In the Sapporo cohort, total mercury levels in maternal hair samples were measured by an oxygen combustion-gold amalgamation method using an atomic absorption detector at the National Institute for Minamata Disease [43, 44].

Phthalate esters and organophosphate flame retardants

In the Sapporo cohort, to determine maternal phthalate exposure levels, MEHP (a metabolite of DEHP) levels in maternal blood were analyzed by GC–MS at Nagoya University [45]. In the Hokkaido cohort, 7 phthalates and 11 organophosphate flame-retardants were measured from dust samples using GC–MS (SIM) analysis. House dust mites were also measured using the ELISA method. The method to analyze 7 phthalate metabolites in urine samples by GC–MS was established, and urine samples from the children were measured to examine the correlation between these metabolites and asthma and allergies [46, 47]. Home visits were also conducted for the children that lived in Sapporo City. During the home visit, house dust and urine samples from the child were collected. In addition, trained researchers evaluated the home interior and dampness.

Bisphenol A

In the Sapporo cohort, Bisphenol A concentrations in maternal and cord blood were analyzed by isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC–MS/MS) at IDEA Consultants, Inc. [48].

Cotinine

In the Sapporo and the Hokkaido cohorts, cotinine concentrations in maternal serum were measured using an enzyme-linked immunosorbent assay (ELISA) kit to evaluate smoking exposure levels [49].

Outcome measurements

The Sapporo cohort

In the Sapporo cohort, with the purpose of assessing the neurodevelopment of the children, several behavioral examinations were conducted during each study period. The Bayley Scales of Infant Development second edition (BSID-II) was used at 6–7 and 18 months of age. The

Fagan Test of Infant Intelligence (FTII) was performed to measure visual recognition memory and cognitive ability in infants aged 6–7 months. To examine developmental progress, the Japanese version of the Denver Developmental Screening Tests (DDST) was used at 18 months of age. At 3.5 years of age, child and maternal intelligence was measured using the Japanese version of the Kaufman Assessment Battery for Children (K-ABC) and the Wechsler Adult Intelligence Scale-Revised (WAIS-R), respectively. At 7 years of age, the Wechsler Intelligence Scale for Children third edition (WISC-III) and the Wisconsin Card Sorting Test (WCST-KFS version) were used to assess the intellectual development and executive function of the children [50, 51]. The Evaluation of Environmental Stimulation (EES) was used to investigate the environmental conditions of children at 6, 18 months and 3.5 years of age. The Japanese version of the Child Behavior Checklist (CBCL) was used to collect information on child behavior at age 3.5 and 7 years of age. The check list of play behavior, Pre-School Activity Inventory-Japanese version (PSAI-J), which was translated from the original version of PSAI, was used to assess the play behavior of the children at 7 years of age [52]. In addition, we also obtained the children's medical history from the follow-up questionnaires performed at each study period. The children's medical history contained information pertaining to the development of atopic dermatitis, asthma, allergies, otitis media, pneumonia or bronchitis and chickenpox.

The Hokkaido cohort

In the Hokkaido cohort, the development of allergies at 1, 2, 4 and 7 years of age and neurodevelopmental disorders at 8 years of age were examined in detail. For allergy assessment, follow-up questionnaires were distributed to children aged 1, 2, 4 and 7 years old, which included questions pertaining to asthma and allergies from the ISAAC and ATS-DLD questionnaires [28, 30]. We also obtained the medical history of the children from the follow-up questionnaires during each study period. The medical histories contained information pertaining to the development of atopic dermatitis, asthma, allergies, otitis media, pneumonia or bronchitis, chickenpox, heart disease, hypospadias or cryptorchidism, thyroid gland malfunction, epilepsy and developmental disorders. In addition to the questionnaire survey, mothers were asked to collect house dust and a sample of the child's urine when the child reached 7 years old.

At 8 years of age, a specific follow-up questionnaire was used to assess the development of neurodevelopmental disorders, specifically ADHD. The questionnaire contained questions pertaining to health status including the treatment the subject received for ADHD, the hours of rising and bedtime as a daily rhythm, and the number of hours the subject enjoys audio-visual tools. To assess ADHD, the Conners third Edition-Parent Japanese version (Conners3P) and the ADHD Rating Scale-IV (ADHD-RS-IV) were used. We also used the Pre-School Activities Inventory Japanese version (PSAI-J) to assess the play behavior of the children. The Evaluation of Environmental Stimulation (EES) was used as a questionnaire to assess the subject's home environment [53]. We also assessed any stressful life events of the children by using the Life Event Questionnaire for Parents (Life Event) [54].

After receiving responses from the 8-year questionnaire, additional questionnaires were distributed to collect more information about the family. The additional questionnaire assessed the working status and health of the parents, the mental condition of the mother, and the use or lack of use of supportive education. To assess a child-rearing environment, we asked about the parent's social networks and supports during child rearing. To assess developmental disorders such as Autism and Asperger syndrome, we used the Japanese version of the Autism screening Questionnaire (ASQ). Additional assessments of the children we obtained using the Japanese version of the Child Behavior Checklist (CBCL) and The Wechsler Intelligence Scale for Children fourth edition (WISC-IV). We also used the Parenting Stress Index (PSI) in Japanese.

Genetic analyses

Genes that were already analyzed using the SNP assay are described in Table 2. Genetic polymorphisms were determined by means of the Taq Man (Applied Biosystems, Inc., Foster City, CA, USA) polymerase chain reaction (PCR) method using minor groove binder (MGB) probes. The polymorphisms analyzed thus far are rs4646903 (T > C, MspI) and rs1048963 (A > G, Ile462Val) of *CYP1A1* (cytochrome P450, family 1, subfamily A polypeptide 1), rs762551 (A > C) of *CYP1A2* (CYP1 subfamily A polypeptide 2), rs1056836 (C > G, Leu432Val) of *CYP1B1* (CYP1 subfamily B polypeptide 1), rs2066853 (G > A, Arg554Lys) of *AHR* (aryl hydrocarbon receptor), rs2292596 (C > G, Pro185Ala) of *AHRR* (AHR repressor), rs1800566 (C609T) of *NQO1* (NAD(P)H: quinone oxidoreductase 1), rs3813864 (-1294G/C) of CYP2E1 (CYP2 subfamily E polypeptide 1), rs1801133 (C677T) and rs1801131 (A1298C) of *MTHFR* (methylenetetrahydrofolate reductase). In addition, copy number variations (CNVs) in *GSTM1* (glutathione S-transferase mu-1) and *GSTT1* (glutathione S-transferase theta-1) were also evaluated [55–58].

Table 2 Genetic factors and its environmental interaction being studied in the Hokkaido study (up to 2013)

Maternal genetic factors	Environmental exposure	Outcomes	Results	Ref.
AHR, AHRR, **CYP1A1**, CYP1A2, CYP1B1	Dioxin and dioxin-like PCBs	(Concentration)	Decreased	[55]
AHR, CYP1A1, GSTM1, GSTT1	Active tobacco smoking (PAHs)	Birth size	Reduction	[56]
NQO1, CYP2E1, MGMT	Active tobacco smoking	Birth size	Reduction	[57]
5,10-MTHFR (C677T, A1298C)	Tobacco smoking and Folic acid	Birth weight	Reduction	[58]

Genes described in bold font in the table represent the genetic polymorphisms that are significantly associated with the outcome

AHR aryl hydrocarbon receptor, *AHRR* AHR repressor, *CYP1* cytochrome P450, family 1, *CYP1A1* CYP1 subfamily A polypeptide 1, *CYP1A2* CYP1 subfamily A polypeptide 2, *CYP1B1* CYP1 subfamily B polypeptide 1, *GSTM1* glutathione-*S*-transferase mu-1, *GSTT1* glutathione-*S*-transferase theta-1, *NQO1* NAD(P)H: quinone oxidoreductase 1, *CYP2E1* CYP2 subfamily E polypeptide 1, *MTHFR* methylenetetrahydrofolate reductase

Results

The characteristics of the participants of the Hokkaido study

A total of 514 mothers were registered in the Sapporo cohort, and another 20,940 mothers were registered in the Hokkaido cohort as of the end of April 2012. The profile of the Sapporo cohort and the partial profile ($n = 2{,}777$) of the Hokkaido cohort had been described previously [27]. We also estimated the prevalence of congenital anomalies in the Hokkaido prefecture. Among the 19,680 mothers included in the Hokkaido cohort between 2003 and 2012, there were 378 subjects with congenital anomalies. The most frequent congenital anomaly was congenital heart defects (35.6 per 10,000 persons), followed by cryptorchidism (15.2), down syndrome (12.2), polydactyly (9.7), hypospadias (9.1) and hydronephrosis (7.6). The total prevalence of congenital anomalies was similar to nationwide data reported by the Japan Association of Obstetricians and Gynecologists (JAOG). However, the number of serious cases was less than that of the JAOG since the members of the JAOG are medical universities and tertiary hospitals and they tend to treat pregnant women with severe complications including fetal congenital anomalies, whereas those of our cohort study are general hospitals and clinics.

The effects of PCDD/PCDF and dioxin-like PCB exposure

Birth weight

In the Sapporo cohort, we observed significant negative correlations between the birth weight of all infants and total PCDF levels, total PCDD TEQ, total PCDF TEQ, total PCDD/PCDF TEQ and total TEQ levels in maternal blood during pregnancy after adjustment for potential covariates. Among male infants, significant adverse associations between birth weight and total PCDD TEQ levels, total PCDD/PCDF TEQ levels and total TEQ levels were found. Moreover, we found significant negative association between birth weight and the levels of 2,3,4,7,8-PeCDF (−24.5 g, 95 % CI −387.4 to −61.5) [59] (Table 3).

Table 3 Gender differences in the effect of PCB/dioxins exposure on birth weight in a multiple linear regression model

$\log_{10}$ scale	Male		Female	
	β[a]	p value	β[a]	p value
Total (pg/g lipid)				
Total PCDDs	−125.7	0.371	−19.3	0.890
Total PCDFs	−237.6	0.191	−304.9	0.058
Total PCDDs/PCDFs	−136.6	0.340	−28.7	0.839
Total non-*ortho* PCBs	−90.7	0.491	−122.4	0.286
Total mono-*ortho* PCBs	−138.6	0.244	−104.3	0.315
Total DL-PCBs	−138.7	0.245	−105.3	0.311
Total dioxin	−148.5	0.229	−106.8	0.319
TEQ (WHO 2005)[†] (TEQ pg/g lipid)				
Total PCDDs TEQ	**−331.4**	**0.019***	−126.3	0.336
Total PCDFs TEQ	−269.8	0.070	−241.7	0.058
Total PCDDs/PCDFs TEQ	**−338.7**	**0.022***	−173.9	0.195
Total non-*ortho* PCBs TEQ	−107.3	0.288	−114.8	0.196
Total mono-*ortho* PCBs TEQ	−138.6	0.244	−104.3	0.315
Total DL-PCBs TEQ	−112.1	0.278	−117.5	0.195
Total dioxin TEQ	**−289.5**	**0.037***	−144.2	0.243

This table was reconstructed by using data from a previously published study by Konishi et al. [59]. Among male infants, a significant negative association between birth weight and total PCDDs TEQ levels, total PCDDs/PCDFs TEQ levels and total TEQ levels was found. However, among the female infants, these significant associations were not found

* $p < 0.05$

† The Toxicity Equivalency Quantity (TEQ) levels were calculated by multiplying the levels of individual congeners by its toxic equivalency factor (TEF) values of WHO 2005 [38]

[a] Beta coefficients represent the change in birth weight (g) for a 10-fold increase in the levels of PCDDs/PCDFs and DL-PCBs

Neurodevelopment

In the Sapporo cohort, after adjusting for potential confounding variables, total PCDD, total PCDDs/PCDF and 1,2,3,4,6,7,8-HpCDD levels in maternal blood during pregnancy were significantly negatively associated with the mental developmental index (MDI) of BSID-II at 6 months of age. Total 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF and 1,2,3,6,7,8-HxCDF were significantly negatively associated with the psychomotor developmental index (PDI) of BSID-II at 6 months of age. Our results suggest that a low-level of exposure to several congeners of PCDDs or PCDFs during pregnancy can affect the neurodevelopment of 6-month-old infants [60]. In addition, when we stratified the data by infant sex, the effects of intrauterine exposure to select PCDD, PCDF and PCB congeners on the PDI score in male infants were more significant (Table 4).

Allergy and infectious diseases

In the Sapporo cohort, our results show that dioxins concentrations in maternal blood during pregnancy are only negatively correlated with cord serum IgE levels in male infants [61] (Table 5). Relatively higher levels of PCDFs were associated with a significantly increased risk of otitis media at 18 months of age, among all infants (odds ratio = 2.5, 95 % confidence interval = 1.1–5.9). Relatively higher levels of 2,3,4,7,8-PeCDF were also associated with a significantly increased risk of otitis media (odds ratio = 5.3, 95 % confidence interval = 1.5–19) among male infants (Table 6). However, we observed a weak association between dioxin-like compound levels and allergy symptoms during infancy. At environmental levels, prenatal exposure to dioxin-like compounds may alter immune function and increase the risk of infections in infancy, especially among males. The compound 2,3,4,7,8-PeCDF may be responsible for this [62].

The effects of PFCs exposure

Temporal trends of PFC levels in maternal plasma

In the Sapporo cohort, the concentrations of PFOS and PFOA ranged from 1.3 to 16.2 ng/ml for PFOS and from below the detection limit to 5.3 ng/ml for PFOA (both detection limits were 0.5 ng/ml) in the blood of pregnant women recruited between 2002 and 2005 [63].

In the Hokkaido cohort, between February 2003 and December 2009, 300 women were randomly selected every year, and the concentrations of 11 PFCs were measured in 2,095 maternal plasma samples. A temporal trend in PFC levels from 2003 to 2011 was also examined. The PFOS and PFOA concentrations in the Hokkaido cohort were lower than those of pregnant women in the Sapporo cohort. Additionally, PFUnDA, PFDoDA and PFTrDA levels were higher in the Hokkaido cohort than individuals of foreign countries. Although the values were lower than the values obtained from individuals in other areas of Japan, there was no significant temporal trend [64].

Birth weight

We examined a correlation between maternal serum PFOS and PFOA concentrations and infant birth weight in the Sapporo cohort. A $\log_{10}$-unit increase in PFOS levels correlated with a decrease in birth weight of 148.8 g (95 % CI 297.0–0.5) after adjusting for confounders; however, no correlation was observed between PFOA levels and birth weight. Our results indicate that in utero exposure to relatively low levels of PFOS is negatively correlated with birth weight [63].

In the Hokkaido cohort, the effects of 11 PFCs including PFHxA, PFHpA, PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA in maternal blood obtained during pregnancy were evaluated. After adjusting for possible confounding factors, PFNA levels negatively correlated with birth weight (per ln-unit: partial regression coefficient $\beta = -41.7$ g, 95 % CI, −77.9 to −5.6 g). After gender stratification, PFNA levels negatively correlated with male birth weight (per ln-unit: $\beta = -59.3$ g, 95 % CI, −110.2 to −8.3 g). Additionally, PFUnDA and PFTrDA levels negatively correlated with female birth weight (per ln-unit: $\beta = -42.0$ g, 95 % CI, −84.6 to 0.6 g and $\beta = -44.9$ g, 95 % CI, −90.1 to 0.3 g, respectively).

Allergy and infectious diseases

In the Sapporo cohort, we investigated the relationship between prenatal exposure to PFOS and PFOA and the development of infant allergies and infectious diseases during the first 18 months of life. Additionally, the effects of PFOS and PFOA on cord blood IgE levels were also evaluated. We found a curvilinear relationship between maternal PFOA levels and cord blood IgE levels. Cord blood IgE levels decreased significantly with high maternal PFOA concentrations among female infants. When $\log_{10}$-transformed maternal PFOA levels changed from 0.3 to 0.7 ng/mL, $\log_{10}$-transformed cord blood IgE levels greatly decreased by −0.863 IU/mL. However, there were no significant associations among maternal PFOS and PFOA levels and food allergies, eczema, wheezing or otitis media in the 18-month-old infants after adjustment for potential confounding variables [31].

In the Hokkaido cohort, we investigated the relationship between prenatal exposure to 11 PFCs and infant

Table 4 Gender differences in the effect of PCB/dioxins exposure on BSID-II Mental (MDI) and Psychomotor (PDI) development scores at 6 months of age in multiple linear regression models

(log_{10} transformed)	6 months MDI						6 months PDI					
	Male ($n = 99$)			Female ($n = 91$)			Male ($n = 99$)			Female ($n = 91$)		
	β^a	t	p value	β^a	t	p value	β^a	t	p value	β^a	t	p value
PCDD												
2,3,7,8-TCDD	−0.15	−1.54	0.13	−0.05	−0.48	0.63	**−0.19**	**−2.01**	**0.048***	−0.06	−0.56	0.58
1,2,3,7,8-PeCDD	−0.07	−0.70	0.48	**0.22**	**2.14**	**0.04***	−0.10	−0.98	0.33	−0.04	−0.33	0.75
1,2,3,4,6,7,8-HpCDD	**−0.25**	**−2.52**	**0.01***	−0.14	−1.34	0.18	**−0.24**	**−2.56**	**0.01***	−0.19	−1.78	0.08
OCDD	−0.09	−0.92	0.36	−0.18	−1.74	0.09	**−0.22**	**−2.33**	**0.02***	−0.21	−1.97	0.05
PCDF												
2,3,7,8-TCDF	−0.08	−0.84	0.41	−0.11	−1.05	0.30	**−0.21**	**−2.21**	**0.03***	−0.13	−1.21	0.23
1,2,3,7,8-PeCDF	−0.02	−0.22	0.83	−0.06	−0.54	0.59	**−0.22**	**−2.38**	**0.02***	−0.17	−1.59	0.12
1,2,3,4,7,8-HxCDF	−0.07	−0.73	0.47	−0.10	−0.93	0.36	−0.17	−1.69	0.09	**−0.25**	**−2.36**	**0.02***
Non-*ortho* PCB												
33′44′5-PenCB (#126)	−0.03	−0.33	0.74	−0.01	−0.10	0.93	−0.15	−1.62	0.11	**−0.24**	**−2.25**	**0.03***
Mono-*ortho* PCB												
2344′5-PeCB (#114)	−0.07	−0.71	0.48	0.08	0.79	0.43	**−0.19**	**−2.00**	**0.049***	−0.16	−1.49	0.14
2′344′5-PeCB (#123)	0.02	0.23	0.82	0.01	0.05	0.96	−0.13	−1.39	0.17	**−0.25**	**−2.37**	**0.02***
233′44′5′-HxCB (#157)	−0.08	−0.85	0.40	0.10	0.90	0.37	**−0.21**	**−2.19**	**0.03***	−0.11	−1.09	0.28
23′44′55′-HxCB (#167)	−0.05	−0.49	0.63	0.04	0.41	0.69	**−0.22**	**−2.35**	**0.02***	−0.15	−1.38	0.17
Di-*ortho* PCB												
22′33′44′5-HpCB(#170)	−0.13	−1.25	0.22	0.10	0.88	0.38	**−0.25**	**−2.47**	**0.02***	−0.04	−0.37	0.71
22′344′55′-HpCB(#180)	−0.13	−1.23	0.22	0.10	0.88	0.38	**−0.24**	**−2.42**	**0.02***	0.00	0.01	1.00
Total												
Total PCDD	−0.10	−1.00	0.32	−0.17	−1.63	0.11	**−0.22**	**−2.31**	**0.02***	−0.21	−1.97	0.05
Total PCDF	−0.06	−0.61	0.55	0.02	0.15	0.88	−0.18	−1.81	0.07	−0.20	−1.83	0.07
Total PCDD/PCDF	−0.10	−1.00	0.32	−0.17	−1.58	0.12	**−0.22**	**−2.33**	**0.02***	−0.21	−1.98	0.05
Total non-*ortho* PCBs	−0.01	−0.12	0.91	0.03	0.25	0.81	−0.16	−1.72	0.09	−0.19	−1.73	0.09
Total mono-*ortho* PCBs	−0.05	−0.55	0.58	0.05	0.46	0.64	−0.19	−1.97	0.05	−0.17	−1.60	0.11
Total DL-PCB	−0.05	−0.55	0.59	0.05	0.46	0.65	−0.19	−1.97	0.05	−0.17	−1.60	0.11
Total dioxins	−0.06	−0.56	0.58	0.04	0.39	0.70	**−0.19**	**−2.03**	**0.045***	−0.17	−1.65	0.10
Total PCDD-TEQ†	−0.09	−0.87	0.39	0.14	1.31	0.19	−0.12	−1.24	0.22	−0.08	−0.77	0.44
Total PCDF-TEQ†	−0.03	−0.28	0.78	0.08	0.73	0.47	−0.17	−1.74	0.09	−0.15	−1.42	0.16
Total PCDD/PCDF-TEQ†	−0.08	−0.75	0.45	0.13	1.19	0.24	−0.14	−1.39	0.17	−0.10	−0.95	0.34
Total non-*ortho* PCBs-TEQ†	−0.03	−0.28	0.78	0.01	0.08	0.94	−0.16	−1.67	0.10	**−0.22**	**−2.04**	**0.04***
Total mono-*ortho* PCBs-TEQ†	−0.05	−0.55	0.58	0.05	0.46	0.64	−0.19	−1.97	0.05	−0.17	−1.60	0.11
Total DL-PCB-TEQ†	−0.05	−0.55	0.59	0.05	0.46	0.65	−0.19	−1.97	0.05	−0.17	−1.60	0.11
Total dioxins-TEQ†	−0.05	−0.53	0.60	0.09	0.84	0.41	−0.15	−1.52	0.13	−0.15	−1.39	0.17

This table was constructed by reanalyzing the data from a previous study by Nakajima et al. [60]. Only statistically significant congeners are presented in this table (*$p < 0.05$)

† The TEQ levels were calculated by multiplying the levels of individual congeners by its TEF values of WHO 2005 [38]

allergies during the first 12 months of life. The characteristics of the participants and information pertaining to infant allergies were obtained from a baseline questionnaire administered to the mother during pregnancy, medical records from the time of delivery and a follow-up questionnaire when the child was 12 months of age. The risk of eczema, wheezing and food allergies during the first 12 months of life was not associated with maternal levels of 11 PFCs, including longer-chain compounds. Odds ratios for eczema and wheezing ranged from 0.66 to 0.73 and from 0.60 to 0.81 for the three higher quartiles of maternal PFTrDA levels, compared with the lowest in the adjusted models, but no dose–response pattern was found [65].

Table 5 Gender differences in the effect of PCB/dioxins exposure on cord blood IgE level in multiple linear regression models

(log_{10} transformed)	Male ($n = 112$)				Female ($n = 123$)			
	Crude model		Adjusted model		Crude model		Adjusted model	
	β (95 % CI)	p value	β (95 % CI)	p value	β (95 % CI)	p value	β (95 % CI)	p value
Total								
Total PCDD	0.032 (−0.681, 0.746)	0.928	−0.061 (−0.821, 0.700)	0.875	0.562 (−0.164, 1.287)	0.128	0.594 (−0.198, 1.386)	0.140
Total PCDF	−0.630 (−1.503, 0.244)	0.156	**−1.097** (−2.127, −0.067)	**0.037****	0.455 (−0.350, 1.261)	0.265	0.590 (−0.313, 1.493)	0.198
Total PCDD/PCDF	0.012 (−0.715, 0.740)	0.973	−0.088 (−0.866, 0.689)	0.822	0.571 (−0.164, 1.306)	0.127	0.607 (−0.195, 1.410)	0.136
Total non-*ortho* PCBs	−0.201 (−0.811, 0.410)	0.516	−0.587 (−1.305, 0.132)	0.108	0.383 (−0.154, 0.919)	0.16	0.479 (−0.110, 1.067)	0.110
Total mono-*ortho* PCBs	−0.252 (−0.804, 0.299)	0.367	−0.482 (−1.137, 0.172)	0.147	0.120 (−0.366, 0.605)	0.626	0.230 (−0.330, 0.790)	0.418
Total DL-PCB	−0.253 (−0.805, 0.300)	0.367	−0.484 (−1.140, 0.171)	0.146	0.121 (−0.365, 0.607)	0.622	0.232 (−0.329, 0.792)	0.415
Total dioxins	−0.246 (−0.817, 0.325)	0.395	−0.521 (−1.275, 0.234)	0.174	0.142 (−0.346, 0.631)	0.566	0.375 (−0.219, 0.970)	0.214
TEQ (WHO 2005)[†]								
Total PCDD TEQ	**−0.630** (−1.288, 0.028)	**0.060***	**−1.008** (−1.822, −0.194)	**0.016****	0.138 (−0.453, 0.728)	0.645	0.332 (−0.376, 1.039)	0.355
Total PCDF TEQ	**−0.689** (−1.408, 0.030)	**0.060***	**−1.229** (−2.113, −0.344)	**0.007*****	0.390 (−0.227, 1.007)	0.213	0.643 (−0.065, 1.352)	0.075
Total PCDD/PCDF TEQ	**−0.681** (−1.373, 0.011)	**0.054***	**−1.144** (−2.006, −0.282)	**0.010****	0.203 (−0.406, 0.812)	0.511	0.427 (−0.299, 1.153)	0.246
Total non-*ortho* PCBs TEQ	−0.234 (−0.689, 0.222)	0.312	**−0.498** (−1.017, 0.021)	**0.060***	0.205 (−0.216, 0.627)	0.337	0.251 (−0.217, 0.719)	0.290
Total mono-*ortho* PCBs TEQ	−0.252 (−0.804, 0.299)	0.367	−0.482 (−1.137, 0.172)	0.147	0.120 (−0.366, 0.605)	0.626	0.230 (−0.330, 0.790)	0.418
Total DL-PCB TEQ	−0.242 (−0.708, 0.224)	0.305	**−0.514** (−1.047, 0.019)	**0.058***	0.202 (−0.228, 0.632)	0.354	0.254 (−0.224, 0.732)	0.295
Total dioxins TEQ	−0.535 (−1.176, 0.106)	0.101	**−1.011** (−1.794, −0.229)	**0.012****	0.234 (−0.337, 0.806)	0.419	0.406 (−0.265, 1.076)	0.233

This table was reconstructed by using data from a previously published study by Washino et al. [61]. Multiple linear regression adjusted for mother's age, maternal allergy history, paternal allergy history, smoking during pregnancy, parity, gestational age, frequency of deep sea fish consumption, distance of highway to home and blood sampling period

* $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$

[†] The TEQ levels were calculated by multiplying the levels of individual congeners by their TEF values of WHO 2005 [38]

Table 6 Gender differences in the effect of PCB/dioxins exposure on the onset of otitis media at 18 months of age in multiple logistic regression models

(log_{10} transformed)		Adjusted			*p*-value for trend
		Quartile 2 OR (95 % CI)	Quartile 3 OR (95 % CI)	Quartile 4 OR (95 % CI)	
All					
TEQ†					
PCDDs	∑ PCDDs†	1.2 (0.53–2.7)	1.1 (0.50–2.6)	1.5 (0.65–3.5)	0.39
PCDFs	∑ PCDFs†	1.6 (0.68–3.8)	2.2 (0.93–5.1)	**2.5** (1.1–5.9)*	**0.03**
Non-*ortho* PCBs	∑ Non-*ortho* PCBs†	1.8 (0.79–4.2)	**2.5** (1.1–6.0)*	1.5 (0.62–3.6)	0.30
Total Dioxins†		2.1 (0.92–4.8)	1.7 (0.71–3.9)	1.7 (0.70–4.1)	0.38
Congeners					
PCDDs	OCDD	**3.4** (1.4–8.5)*	**2.8** (1.1–7.0)*	**2.6** (1.0–6.9)*	0.12
PCDFs	2,3,4,7,8-PeCDF	1.6 (0.7–3.9)	2.0 (0.88–4.8)	**2.8** (1.2–6.6)*	**0.02**
Non-*ortho* PCBs	33′44′-TCB(#77)	2.4 (0.99–5.9)	1.4 (0.61–3.3)	**3.4** (1.6–7.3)*	**0.01**
Mono-*ortho* PCBs	233′44′5′-HxCB(#157)	**2.4** (1.0–5.5)*	1.1 (0.43–2.7)	**2.5** (1.1–5.9)*	0.16
Males					
TEQ†					
PCDDs	∑ PCDDs†	0.5 (0.13–1.8)	2.0 (0.65–6.2)	2.9 (0.83–10)	**0.03**
PCDFs	∑ PCDFs†	1.0 (0.28–3.3)	2.9 (0.87–9.8)	**3.8** (1.1–13)*	**0.01**
Non-*ortho* PCBs	∑ Non-*ortho* PCBs†	2.4 (0.70–8.3)	2.9 (0.86–9.7)	3.6 (0.98–13.3)	0.05
Total dioxins		2.1 (0.61–6.9)	2.2 (0.67–7.1)	**4.4** (1.2–16)*	**0.03**
Congeners					
PCDFs	2,3,4,7,8-PeCDF	1.7 (0.48–6.0)	2.9 (0.87–10)	**5.3** (1.5–19)*	**0.01**
Non-*ortho* PCBs	33′44′-TCB(#77)	2.8 (0.85–9.4)	0.9 (0.24–3.4)	**3.5** (1.2–11)*	0.08
	33′44′55′-HxCB(#169)	1.0 (0.25–3.8)	3.0 (0.93–9.6)	**3.6** (1.1–12)*	**0.01**
Mono-*ortho* PCBs	2344′5-PeCB(#114)	2.4 (0.62–8.9)	**4.5** (1.2–16.6)*	**4.9** (1.3–18)*	**0.01**
	23′44′55′-HxCB(#167)	3.1 (0.83–11)	3.3 (0.91–11)	**3.7** (1.0–13)*	0.06
	233′44′5′-HxCB(#157)	**4.5** (1.2–17)*	1.6 (0.37–6.5)	**7.5** (1.9–29)*	**0.02**
Female					
TEQ†					
PCDDs	∑ PCDDs†	2.3 (0.71–7.6)	0.5 (0.11–2.0)	1.1 (0.30–4.1)	0.44
PCDFs	∑ PCDFs†	**4.0** (1.1–14.7)*	1.2 (0.30–5.1)	1.3 (0.29–5.8)	0.41
Non-*ortho* PCBs	∑ Non-*ortho* PCBs†	1.3 (0.41–4.3)	1.9 (0.51–7.1)	0.8 (0.22–3.1)	0.86
Total Dioxins†		2.6 (0.78–8.6)	1.0 (0.25–4.0)	1.0 (0.27–4.1)	0.57
Congeners					
Non-*ortho* PCBs	33′44′-TCB(#77)	1.4 (0.3–6.9)	1.5 (0.45–4.9)	**3.8** (1.2–12)*	**0.03**

This table was reconstructed by using data from a previously published study by Miyashita et al. [62]. Only statistically significant congeners are presented in this table. (* $p < 0.05$). The OR (95 % CI) versus the first quartile (reference) in the logistic regression model was adjusted for maternal educational level, parity, infant gender, duration of breast-feeding, environmental tobacco exposure, day care attendance and blood sampling period (infant gender was excluded from covariates in gender-stratified analysis)

[a] quartiles applied as ordinal variables in the model

* $p < 0.05$, ** $p < 0.01$; Statistically significant, *p*-value

† The TEQ levels were calculated by multiplying the levels of individual congeners by its TEF values of WHO 2005 [38]

Gene–environment interaction

The effects of maternal genetic polymorphisms on dioxin concentration

Dioxins are metabolized by cytochrome P450, family 1 (CYP1) via AHR. We determined whether different blood dioxin concentrations are associated with polymorphisms in the *AHR* (dbSNP ID: rs2066853), the *AHRR* (rs2292596), the *CYP1A1* (rs4646903 and rs1048963), the *CYP1A2* (rs762551) and the *CYP1B1* (rs1056836) in pregnant Japanese women. Comparisons between the GG, GA and AA genotypes of the *AHR* showed a significant difference for both the mono-*ortho* PCBs concentrations (genotype model; GG:GA:AA = 11,266.3:13,146.5:12,948.9 (pg/g lipid), $p = 0.016$) and that of toxicity equivalence quantities [TEQs] (GG:GA:AA = 0.338:0.394:0.388 (TEQ pg/g lipid), $p = 0.016$). Second, we found a significant

association with the dominant genotype model for the PCDDs TEQs ([TT + TC]:CC = 7.408:6.480 (TEQ pg/g lipid), $p = 0.048$) and for PCDFs TEQs ([TT + TC]:CC = 2.596:2.267 (TEQ pg/g lipid), $p = 0.035$) 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 [55].

Genetic polymorphisms and maternal smoking

The effects of maternal smoking and genetic polymorphisms on infant birth size were examined in the Sapporo cohort. Birth weight and length were significantly lower among infants born to smokers with the *AHR* GG genotype, the *CYP1A1* TC/CC genotype or the *GSTM1* null genotype. When combinations of these genotypes were considered, birth weight and length were significantly lower for infants of continuously smoking women with the *AHR* GG genotype and CYP1A1 TC/CC genotype (−315 g and −1.7 cm, respectively) and with the *CYP1A1* TC/CC genotype and *GSTM1* null genotype (−237 g and −1.3 cm, respectively) [56] (Fig. 4). For polymorphisms in the gene-encoding N-nitrosamine-metabolizing enzymes, *NQO1*, birth weight, birth length and birth head circumference were significantly reduced (−199 g, −0.8 cm and −0.7 cm, respectively) among infants born to smokers with the *NQO1* CC genotype (Fig. 5). This genotype did not confer adverse effects among women who had never smoked or who quit smoking during the first trimester. Our results suggest an important modifying role of polymorphisms in metabolizing enzyme genes in concert with the adverse effects of maternal smoking on infant birth size [57].

Folate, maternal smoking and genetic polymorphisms

Folate is essential for fetal growth and development, and smoking has been associated with nutritional deficiencies in vitamins including folate. The birth weight of infants born to moderate smokers (≥10 cigarettes per day) with low folate status (<6.0 ng/ml) was lower by 107 g compared with non-smokers having a normal folate status (≥6.0 ng/ml). Maternal 5,10-methylenetetrahydrofolate reductase (*MTHFR*) 1298AA was associated with low folate status. The 5,10-*MTHFR* AA genotype was associated with a decrease in birth weight by 107 g in infants born to smokers. After stratification by infant gender, the effect was more pronounced in male infants with a reduction in birth weight of 117 g. Female infants never demonstrated any statistically significant changes in birth weight [58].

Discussion

What are the primary strengths and weaknesses of the study?

The design of our study is a prospective cohort study intended to collect data on environmental exposures during fetal development and to control for potential confounders.

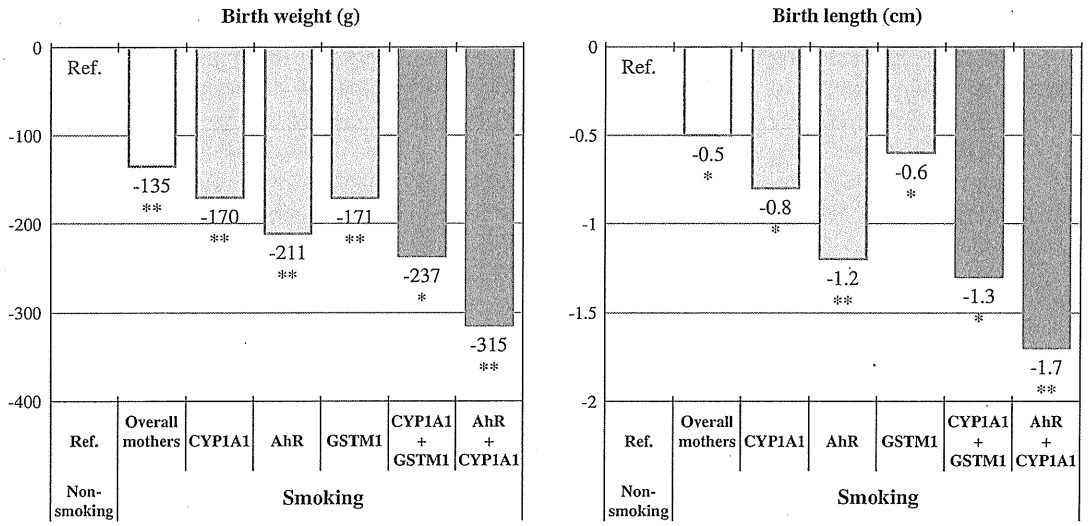

Fig. 4 The effects of maternal smoking in combination with maternal PAHs-metabolism-related genetic polymorphisms on infants' birth size. Adjusted for maternal age, height, weight before pregnancy, alcohol consumption during pregnancy, history of delivery, newborn sex, gestational weeks and household income. $*p < 0.05$, $**p < 0.01$ This figure was created by modifying a figure contained in our previous study by Sasaki et al. [56]

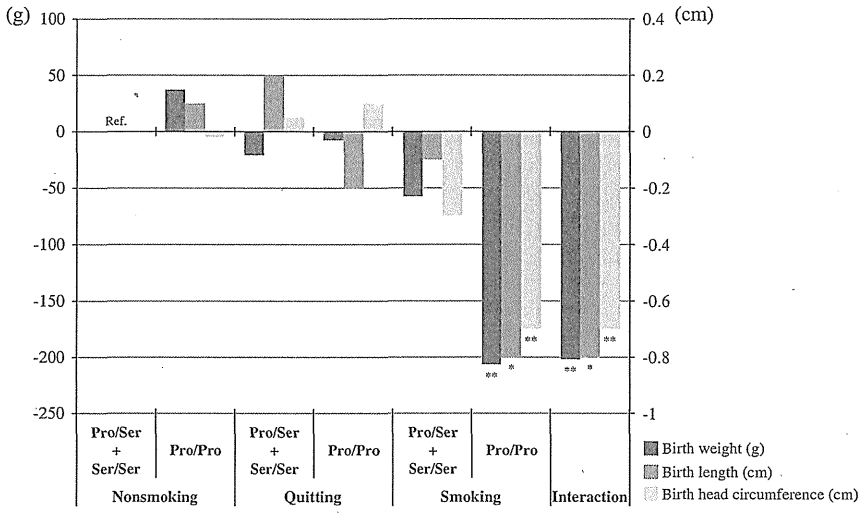

Fig. 5 The effects of maternal smoking in combination with maternal NQO1 genotype on infants' birth size. Adjusted for maternal age, height, weight before pregnancy, weight gain during pregnancy, alcohol consumption during pregnancy, parity, infant gender, gestational age, and household income. Interaction in multiple linear regression models was defined as product terms for the product of the dummy independent variables: maternal smoking status (nonsmoker, quitter or smoker) and genotype (wild or mutant). β represents the product term for smoker × wild genotype. *NQO1* NAD(P)H: quinone oxidoreductase 1, *Pro* proline, *Ser* serine This figure was constructed by using the data from previous study by Sasaki et al. [57]

The detailed measurements in exposures and outcomes are adequate to detect the various effects of perinatal environmental and genetic determinants on childhood outcomes. In the Sapporo cohort study, face-to-face examinations for neurodevelopment assessment were conducted. The Hokkaido cohort had been the largest birth cohort in Japan until 2011 when the nation-wide cohort study, the Japan Environment and Children's Study (JECS), was launched based upon our study design. A potential problem of our study is that both the Sapporo and Hokkaido cohorts may have been biased in participant selection because they are both hospital-based studies, although the latter cohort consists of the hospitals and clinics over the Hokkaido areas to mitigate that bias (Fig. 1). In addition, despite our efforts to keep track of participants' residence with periodical newsletters, some levels of attrition were caused by individuals moving outside of the study area.

The main findings of the study

Over the last decade, we have been intensely investigating the effects of intrauterine chemical exposures on children's health. The main findings of our study are as follows.

1. The effects of dioxins—with emphasis on gender differences

We discovered that there are gender differences in the effects of dioxins and DL-PCBs on birth weight, infants' neurodevelopment and immune functions; our results suggest that the male infants are more susceptible to those chemicals than female infants. Our observations on birth weight were in concordance with other studies, which indicated a stronger negative effect of these compounds on the birth weight of male infants [66–70]. In our study, we found that the adjusted regression coefficients of total PCDDs TEQ and PCDDs/PCDFs TEQ levels among male and female infants were −331.4 and −126.3 g and −338.7 and −173.9 g, respectively. It is possible that male infants had lower birth weights at higher PCDDs and PCDDs/PCDFs TEQ levels in the maternal blood than female infants. In addition to birth weight, we also found that dioxin-like compounds had negative effects on neurodevelopment at 6 months of age in addition to the negative effects on infants' immune function such as cord blood IgE levels and otitis media at 18 months of age. Although there are few epidemiological studies examining the effects of intrauterine exposure to dioxin-like compounds that specifically examined gender differences other than birth weight, it appears that male infants are more susceptible to exposure to these chemicals, which might be due to gender-specific endocrine activities. However, examining gender difference in the effects of PCBs and PCDDs/PCDFs are part of a larger discussion on endocrine disruption; therefore, we need more evidence from larger studies with exposure measurements. Recently, we analyzed sex hormone concentrations in cord blood and its correlation with intrauterine EDCs exposure. In the near future, we will be able to examine gender-specific responses to EDCs and their effect on sex hormone levels.

In addition to further epidemiological studies, molecular biological studies using animal models and human cell lines are also necessary to elucidate the molecular mechanisms of gender-dependent susceptibility to the exposures.

2. The different effects of dioxin congeners

We discovered that the different dioxin congeners had different effects on children exposed in utero. Identification of the potent biological properties of PCDDs, PCDFs and DL-PCBs, and which individual congeners of PCDDs, PCDFs and DL-PCBs affect birth outcomes has been an important goal in investigating the mechanism of effect to prevent harmful effects on fetuses. We found negative associations between maternal PCDF and PCDD exposure levels and birth weight and motor development at 6 months of age, and an increased risk of developing otitis media at 18 months of age correlated with maternal PCDF exposure.

In the study of Yu-Cheng children, it was indicated that the PCDFs group, including the penta-CDF and hexa-CDF congeners, were primarily responsible for the observed health effects compared to other groups of PCBs/PCDFs congeners [71]. Moreover, 70 % of the toxicity of TEQ was contributed by 2,3,4,7,8-PeCDF in Yusho patients [72]. These observations were in concordance with our results, which indicated a significant negative association between 2,3,4,7,8-PeCDF and birth weight. In addition, we found that maternal 2,3,4,7,8-PeCDF exposure increased the risk of developing otitis media at 18 months of age. These data suggest that 2,3,4,7,8-PeCDF is one of the most dangerous congeners.

Due to its high affinity for the AHR, it was suggested that there is a specific accumulation of PCDDs and PCDFs congeners including 2,3,4,7,8-PeCDF in the placenta [73, 74], which plays an important role in transporting nutrients and oxygen through cord blood in the developing fetus. Taking the above considerations into account, we suggest that PCDDs and PCDFs congeners, especially 2,3,4,7,8-PeCDF, may accumulate in the placenta and retard important placental functions, which may result in lower birth weight.

We also found significant negative associations between motor development and maternal exposure to isomers of PCDDs and PCDFs and mental development and exposure to levels of total PCDDs and PCDFs. Currently, there were few human or animal experimental studies that have investigated the association between individual isomer levels of PCBs and dioxins and neurodevelopment. These studies are required to elucidate the mechanisms of action of individual congeners on neurodevelopment.

3. The diverse effects of PFCs exposure

Our results suggest that intrauterine PFCs exposure affects not only fetal growth but also the immune system. In the current study, cord blood IgE levels decreased significantly with high maternal PFOA concentrations in female infants. However, no association was observed between maternal serum PFOS and PFOA concentrations and the occurrence of food allergies, eczema, wheezing and otitis media in their infants during the first 18 months of life. The results of the C8 Health Project showed a significant trend in decreasing IgE levels with increasing PFOA levels in maternal blood samples among females [75]. Our results are consistent with those of that study, even though the concentration of maternal PFOA was lower than that measured in other studies, including the C8 Health Project [75–78]. However, we note that the PFOA levels were not associated with the development of allergies and infectious diseases in infants before 18 months of age. In addition, our result contradicted the results of the Taiwan study, which showed that PFOA levels were positively correlated with cord blood IgE levels only in males [21]. It may be necessary to perform follow-up studies to investigate whether prenatal exposure to PFCs affects immune system development (and address potential gender-specific differences) from infancy to school age because it is difficult to obtain definitive diagnoses for infants.

Moreover, a recent result from a prospective cohort study suggested that intrauterine exposure to PFCs could also modulate infants' thyroid hormone levels [19]. They reported that there were significant negative correlations between maternal PFOS and fetal T3, and maternal PFTrDA and fetal T4 and T3 after adjusting for major covariates. However, this was the only epidemiological report regarding prenatal PFCs exposure and infants' thyroid function, and their sample size was insufficient. Thus, we need additional epidemiological studies to validate the effects of intrauterine PFCs exposure on thyroid functions.

In addition, the temporal trends of PFCs levels indicates that PFOS and PFOA concentrations were decreasing every year from 2003 to 2011 due to the restriction of PFOS by the Stockholm Convention on Persistent Organic Pollutants in 2009. Instead, PFNA and PFDA, which have a longer carbon chain than PFOA and are harder to be metabolized in the body, were increasing. Further studies must be conducted to estimate the effects of intrauterine exposure to long-chained PFCs on children's health and development.

4. Genetic susceptibility to the exposures

In our study, we found that the maternal genetic polymorphisms in *AHR* or *CYP1A1* independently modified dioxin concentrations in maternal blood, suggesting different dioxin accumulation in the body of individuals with these genotypes, which would lead to different dioxin exposure levels [55]. *CYP1A1* activation mediated by AHR

is an important mechanism for metabolizing dioxins. Dioxins such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) are sensitive to AHR, and TCDD mediates transcriptional regulation of AHR via its binding with AHR nuclear translocator. Activated AHR facilitates the expression of *CYP1A1*, *CYP1A2*, *CYP1B1* and *AHRR*, which are important for metabolizing dioxins [79]. Moreover, the expression of *CYP1A1* and *CYP1B1* are important for endocrine signaling pathways. Those proteins 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) [80].

In addition to the dioxin concentrations, among the polymorphism groups of *CYP1A1*, *AHR*, *GSTM1* and *NQO1*, we observed different susceptibilities with respect to the effect of maternal smoking exposure on birth size [56, 57]. The AHR, CYP1A1 and GSTM1 metabolize the polycyclic aromatic hydrocarbon (PAH) in tobacco smoke. The GSTM1 detoxifies specific biologically active metabolites of PAHs, and carriers of the *GSTM1* null genotype have a reduced ability to detoxify these metabolites. Our study shows that infants born to mothers that have the *AHR* wild genotype and continuously smoke had a significantly lower birth weight and length compared with infants born to non-smokers; moreover, smokers who had the *AHR* wild type and *CYP1A1* variant genotype had the greatest reduction in both birth weight and length. Because there have only been a few epidemiological studies, further studies are required to clarify the role of the Arg554Lys polymorphism in fetal development.

The NQO1 is an important enzyme that functions in both phase I (activation) and phase II (detoxification) metabolism of xenobiotics depending on the substrate. As a detoxification enzyme, it catalyzes the two-electron reduction of quinoid compounds to the readily excreted hydroquinones to prevent the generation of reactive oxygen species and, thereby, protect cells against oxidative damage. It also catalyzes the activation of some pro-carcinogens such as nitrosamines and heterocyclic amines, which are present in tobacco smoke [81]. Our study suggests an important role for polymorphisms in the *N*-nitrosamine-metabolizing enzyme gene *NQO1* in mitigating the adverse effects of maternal smoking on infant birth size. These findings could have significant public health implications regarding the need for smoking prevention and cessation programs aimed specifically at susceptible women of childbearing age.

In addition, our current results suggest that the adverse health effects of prenatal tobacco smoke exposure resulted not only from active smoking but also from secondhand smoke (SHS) exposure during pregnancy. Birth weight and infant length among SHS-exposed women with the *CYP1A1**2C AG/GG genotypes (−88 g and −0.9 cm, respectively) and the epoxide hydrolase 1 (*EPHX1*) His/His genotypes (−154 g and −1.1 cm, respectively) were significantly lower. The N-acetyl transferase 2 (*NAT2**7) slow acetylators group was also adversely affected (−51 g). A combination of *EPHX1* His/His+*NAT2**7 slow alleles not only resulted in a remarkable decrease in birth weight and length (−145 g and −1.1 cm, respectively) but also demonstrated significant interaction with SHS exposure [82].

The future challenges of the study

1. Inferences from previous studies in hypospadias–gene–environment interactions

As described in our previous review, both genetic and environmental factors contribute to the etiology of congenital malformations such as hypospadias and cryptorchidism [26]. The etiology of hypospadias was unclear in a majority of cases, but it was regarded as a complex disorder caused by both genetic and environmental factors (Fig. 6). Because the development of the urethral and external genital system in the male fetus is androgen-dependent, abnormalities in the synthesis and metabolism of androgens caused by exposure to EDCs can result in abnormal genital developmental phenotypes.

In previous studies, we had clarified the etiology of hypospadias with genetic factors that were related to fetal endocrine activity such as the *ESR1* and *ESR2* and *17βHSD3* and maternal hormonal activity such as the *CYP1A1* in a retrospective case–control study [23, 24, 26]. Hypospadias is a common congenital anomaly caused by an incomplete fusion of the urethral folds. The urethral opening is on the ventral surface of the penis, on the scrotum or the perineum. Thus far, an increase in the prevalence of hypospadias has been reported in various countries, and these trends are speculated to be related to EDC exposure [83]. Several studies have shown the association between hypospadias and fetal gene polymorphisms in genes involved in androgen metabolism [84–86].

These results suggest that environmental factors, including EDCs exposure in utero, as well as genetic factors are responsible for the etiologies of congenital malformations, diseases and birth outcomes such as birth size. Moreover, considering that environmental exposures in utero might affect the children's birth outcomes, the mother's EDCs exposure level and genetic factors that may affect the intrauterine environment are also important factors to consider in evaluating the cause of adverse birth outcomes. Thus, to elucidate the etiology of the disease, two different study approaches must be conducted; one is the screening for genetic risk factors in children and mothers, and the other is to estimate the effect of the environmental risk factors including EDC exposures. In

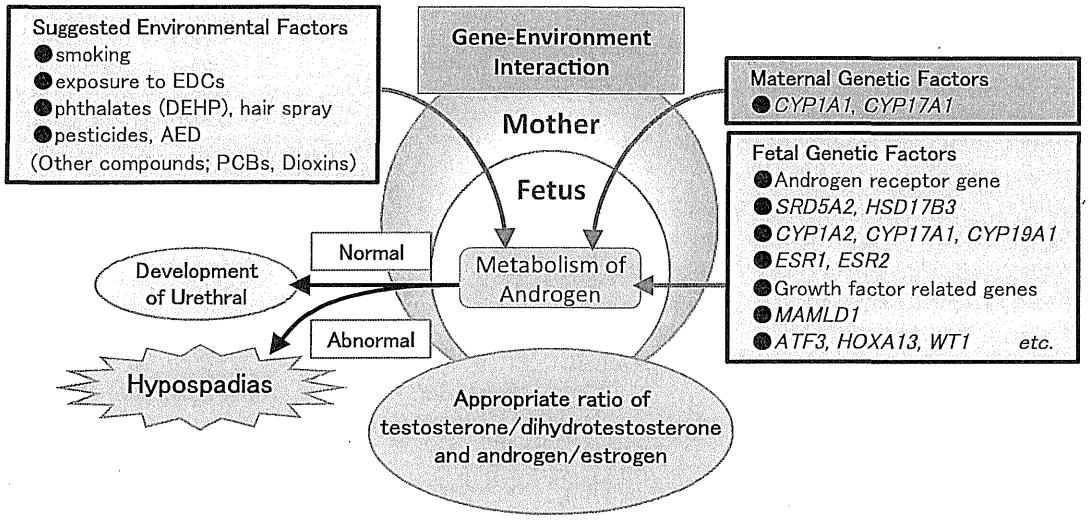

Fig. 6 Summary and suggestions for further studies on the environmental and genetic factors that influence hypospadias development. This figure was created by modifying a figure contained in our previous review by Kishi et al. [26]

addition, by integrating those two approaches to study gene–environment interaction, it becomes possible to identify more susceptible individuals in the population.

2. Gene–environment interactions involved in the etiology of ADHD

In recent years, the increased prevalence of developmental disorders such as Autism Spectrum Disorder (ASD) and ADHD are of increasing concern to the public. Although it is estimated that genetic effects account for 80 % of ASD cases and 79 % of ADHD cases, respectively [87], environmental factors such as the nursing environment and exposure to tobacco smoke also appear to be important factors because the prevalence of these diseases continues to increase while the genetic background of the population remains relatively stable. To date, postnatal environmental exposures, such as passive smoke exposure, iron deficiency, thyroid dysfunction, otitis media and psychosocial stress, are reported as risk factors for ADHD. In addition, prenatal risk factors such as maternal smoking, maternal alcohol intake, lead, PCBs and food additive exposure are also reported to be risk factors for ADHD [88]. Additionally, several studies have indicated that children who were born prematurely or with low birth weight had an increased risk of developing ADHD [89–92], which suggests that the intrauterine environment may play some role. However, the detailed mechanisms of the etiology of those neurodevelopmental disorders have yet to be identified. In the future, by taking genetic and environmental study approaches and studying gene-environment interactions, it is anticipated that all possible risk factors will be elucidated, and eventually, the etiologies of developmental disorders such as Autism and ADHD will be known.

In the present cohort studies, we discovered the maternal genetic factors that affect a child's birth outcome along with the risks associated with maternal smoking and intrauterine dioxin exposure. However, there are few genetic risk factors that have been found thus far considering the large, intricate gene networks involved in a child's health and development. Further studies including genome wide analysis are needed to elucidate the effects of gene–environment interactions.

3. The role of epigenetics

Recently, there has been a growing interest in understanding the role of epigenetics in linking a child's intrauterine environment to future health and disease. Epigenetic modifications, such as DNA methylation, are programmed in utero and are likely to be maintained through cell division and throughout cell linages [93]. Therefore, it is postulated that epigenetic regulation is the "missing link" in the DOHaD hypothesis, which would connect the intrauterine environment to postnatal phenotypes. To date, dozens of animal studies and several epidemiological studies have been conducted to estimate the effect of maternal smoking, environmental chemical exposure and metal exposure in utero on a child's epigenome [94–96]. For instance, maternal smoking exposure increases the methylation of the regulatory region of Insulin-like Growth Factor 2 *(IGF2)* in cord blood DNA, which negatively correlates with IGF2 protein levels in the cord blood [97, 98]. However, at this moment, the epigenetic effects of intrauterine exposure to environmental chemicals are controversial. Further studies

exploring the environmental and genetic risk factors for epigenetic vulnerability is necessary. Currently, we are conducting epigenetic research to investigate the effect of intrauterine exposures to environmental chemicals on a child's epigenome and the resulting risk for future health and disease complications.

As Barker first suggested, the consequences of a disrupted intrauterine environment might be expressed as adverse health outcomes a decade more or later. To thoroughly estimate the effects of intrauterine EDCs exposures in humans, it is necessary to follow individuals in a prospective birth cohort study with a sufficient sample size for a long period.

Working toward international collaboration

In recent years, there has been an avid movement toward collaborating and integrating existing birth cohort studies across borders. The primary purpose of these birth cohort consortiums are to obtain evidence based results by using data from larger sample sizes (meta-analysis), as well as obtaining more applicable and generalizable results by integrating data beyond regions, countries and ethnicities. For instance, in Europe, the Environmental Health Risks in European Birth Cohorts (ENRIECO) was established in 2009 [99]. In Asia, the Birth Cohort Consortium of Asia (BiCCA) is now calling for participation to all existing Asian birth cohorts [http://www.bicca.org]. Although there are many challenges regarding coordination of different cohort studies, we do believe that it is a worthy endeavor.

Additional information concerning the Hokkaido study is available at the study website: http://www.cehs.hokudai.ac.jp/. All of the source data that have been collected are maintained and stored at Hokkaido University Center for Environmental and Health Sciences. Initial approaches or enquiries regarding the study can be made to the principal investigator (rkishi@med.hokudai.ac.jp).

Acknowledgments We would like to express our appreciation to all of the study participants of The Hokkaido Study on Environment and Children's Health. We express our profound gratitude to all personnel in the hospitals and clinics that collaborated with the study: Sapporo Toho Hospital, Keiai Hospital, Endo Kikyo Maternity Clinic, Shiroishi Hospital, Memuro Municipal Hospital, Aoba Ladies Clinic, Obihiro-Kyokai Hospital, Akiyama Memorial Hospital, Sapporo Medical University Hospital, Hokkaido University Hospital, Kitami Red Cross Hospital, Hoyukai Sapporo Hospital, Gorinbashi Hospital, Hashimoto Clinic, Asahikawa Medical College Hospital, Hakodate Central General Hospital, Ohji General Hospital, Nakashibetsu Municipal Hospital, Sapporo Tokushukai Hospital, Asahikawa Red Cross Hospital, Wakkanai City Hospital, Kushiro Rosai Hospital, Sapporo-Kosei General Hospital, Shibetsu City General Hospital, Nikko Memorial Hospital, Sapporo City General Hospital, Kohnan Hospital, Hakodate City Hospital, Hokkaido Monbetsu Hospital, Tenshi Hospital, Hakodate Goryoukaku Hospital, Nakamura Hospital, Kin-ikyo Sapporo Hospital, Kitami Lady's Clinic, Engaru-Kosei General Hospital, Kushiro Red Cross Hospital, Nayoro City General Hospital and Obihiro-Kosei General Hospital. We also deeply express our gratitude to all of the staff of The Hokkaido Study on Environment and Children's Health for their considerable efforts to support our study: Y. Tanabe, M. Kato, Y. Okada, K. Tanaka, M. Hoshiba, N. Takeshima and M. Kihara. This study was funded by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Health, Labor and Welfare; the Ministry of Education, Culture, Sports, Science and Technology; the Japan Society for the Promotion of Science; and the Ministry of Environment.

Conflict of interest None declared.

Appendix: Members of The Hokkaido Study on Environment and Children's Health

S. Tajima, H. Goudarzi, K. Azumi, A. Kanazawa, Y. Otake, T. A. Yila (Hokkaido University Center for Environmental and Health Sciences, Sapporo, Japan), Y. Ait Bamai, S. Cong, Tos. Baba, T. S. Braimoh, S. Ban, N. Washino, K. Konishi, S. Kato, A. Uno, M. Limpar (Department of Public Health Sciences, Hokkaido University Graduate School of Medicine, Sapporo), H. Minakami (Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine, Sapporo), K. Nonomura (Department of Renal and Genitourinary Surgery, Hokkaido University Graduate School of Medicine, Sapporo), T. Mitsui (Department of Urology, Hokkaido University Graduate School of Medicine, Sapporo), T. Endo, Tsu. Baba (Sapporo Medical University, Sapporo), K. Sengoku, Y. Saijo, E. Yoshioka, T. Miyamoto (Asahikawa Medical University, Asahikawa), M. Yuasa (Juntendo University, Tokyo), F. Sata (Department of Epidemiology, National Institute of Public Health, Wako), N. Kurahashi (Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo), J. Tamaki (School of Medicine, Kinki University), J. Kajiwara, T. Todaka (Fukuoka Prefectural Institute of Health and Environmental Sciences, Fukuoka), H. Murohashi (Graduate School of Education, Hokkaido University, Sapporo), H. Matsuura (Laboratory of Bioorganic Chemistry, Division of Applied Bioscience, Research Faculty of Agriculture, Hokkaido University, Sapporo), T. Matsumura (IDEA Consultants,Inc., Shizuoka), M. Ishizuka (Laboratory of Toxicology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo).

Collaborating Institutions

Hokkaido University Center for Environmental and Health Sciences; Hokkaido University Graduate School of Medicine: Departments of Public Health Sciences, Obstetrics and

Gynecology, Pediatrics, Renal and Genitourinary Surgery, Respiratory Medicine and Dermatology; Hokkaido University Graduate School of Veterinary Medicine: Department of Environmental Veterinary Sciences; Hokkaido University Graduate School of Agricultures; Sapporo Medical University: Obstetrics and Gynecology; Asahikawa Medical College: Department of Health Sciences, Obstetrics and Gynecology; Sapporo City Institute of Public Health; Hokkaido Association of Obstetricians and Gynecologists; Fukuoka Institute of Health and Environmental Sciences; Hoshi University School of Pharmacy and Pharmaceutical Sciences, Department of Analytical Chemistry; IDEA Consultants, Inc., Sizuoka; Chubu University, Nagoya.

References

1. Colborn T, Dumanoski D, Myers JP. Our stolen future: are we threatening our fertility, intelligence, and survival? A scientific detective story. New York: Plume; 1997.
2. Wigle DT, Arbuckle TE, Turner MC, Berube A, Yang Q, Liu S, et al. Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. J Toxicol Environ Health B Crit Rev. 2008; 11(5–6):373–517.
3. Schug TT, Janesick A, Blumberg B, Heindel JJ. Endocrine disrupting chemicals and disease susceptibility. J Steroid Biochem Mol Biol. 2011;127(3–5):204–15.
4. Meeker JD. Exposure to environmental endocrine disruptors and child development. Arch Pediatr Adolesc Med. 2012;166(6):E1–7.
5. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet. 1986;1(8489):1077–81.
6. Gluckman P, Hanson M. Developmental origins of health and disease. New York: Cambridge University Press; 2006.
7. Newman J, Ross M. Early life origins of human health and disease. Basel: Karger; 2009.
8. Dietert RR. Developmental immunotoxicology: focus on health risks. Chem Res Toxicol. 2009;22(1):17–23.
9. El Majidi N, Bouchard M, Gosselin NH, Carrier G. Relationship between prenatal exposure to polychlorinated biphenyls and birth weight: a systematic analysis of published epidemiological studies through a standardization of biomonitoring data. Regul Toxicol Pharmacol. 2012;64(1):161–76.
10. Boucher O, Muckle G, Bastien CH. Prenatal exposure to polychlorinated biphenyls: a neuropsychologic analysis. Environ Health Perspect. 2009;117(1):7–16.
11. Goodman JE, Kerper LE, Boyce CP, Prueitt RL, Rhomberg LR. Weight-of-evidence analysis of human exposures to dioxins and dioxin-like compounds and associations with thyroid hormone levels during early development. Regul Toxicol Pharmacol. 2010;58(1):79–99.
12. Vreugdenhil HJ, Slijper FM, Mulder PG, Weisglas-Kuperus N. Effects of perinatal exposure to PCBs and dioxins on play behavior in Dutch children at school age. Environ Health Perspect. 2002;110(10):A593–8.
13. Su PH, Huang PC, Lin CY, Ying TH, Chen JY, Wang SL. The effect of in utero exposure to dioxins and polychlorinated biphenyls on reproductive development in eight year-old children. Environ Int. 2012;39(1):181–7.
14. Winans B, Humble MC, Lawrence BP. Environmental toxicants and the developing immune system: a missing link in the global battle against infectious disease? Reprod Toxicol. 2011;31(3): 327–36.
15. Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, et al. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environ Health Perspect. 2007;115(11): 1670–6.
16. Fei C, McLaughlin JK, Tarone RE, Olsen J. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Environ Health Perspect. 2007;115(11):1677–82.
17. Olsen GW, Butenhoff JL, Zobel LR. Perfluoroalkyl chemicals and human fetal development: an epidemiologic review with clinical and toxicological perspectives. Reprod Toxicol. 2009; 27(3–4):212–30.
18. Halldorsson TI, Rytter D, Haug LS, Bech BH, Danielsen I, Becher G, et al. Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study. Environ Health Perspect. 2012;120(5):668–73.
19. Kim S, Choi K, Ji K, Seo J, Kho Y, Park J, et al. Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ Sci Technol. 2011;45(17): 7465–72.
20. Grandjean P, Andersen EW, Budtz-Jorgensen E, Nielsen F, Molbak K, Weihe P, et al. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA. 2012;307(4):391–7.
21. Wang IJ, Hsieh WS, Chen CY, Fletcher T, Lien GW, Chiang HL, et al. The effect of prenatal perfluorinated chemicals exposures on pediatric atopy. Environ Res. 2011;111(6):785–91.
22. World Health Organization. Possible developmental early effects of endocrine disrupters on child health. Geneva: WHO Press; 2012.
23. Ban S, Sata F, Kurahashi N, Kasai S, Moriya K, Kakizaki H, et al. Genetic polymorphisms of ESR1 and ESR2 that may influence estrogen activity and the risk of hypospadias. Hum Reprod. 2008;23(6):1466–71.
24. Sata F, Kurahashi N, Ban S, Moriya K, Tanaka KD, Ishizuka M, et al. Genetic polymorphisms of 17 beta-hydroxysteroid dehydrogenase 3 and the risk of hypospadias. J Sex Med. 2010;7(8): 2729–38.
25. Kurahashi N, Sata F, Kasai S, Shibata T, Moriya K, Yamada H, et al. Maternal genetic polymorphisms in CYP1A1, GSTM1 and GSTT1 and the risk of hypospadias. Mol Hum Reprod. 2005; 11(2):93–8.
26. Kishi R, Sata F, Yoshioka E, Ban S, Sasaki S, Konishi K, et al. Exploiting gene-environment interaction to detect adverse health effects of environmental chemicals on the next generation. Basic Clin Pharmacol Toxicol. 2008;102(2):191–203.
27. Kishi R, Sasaki S, Yoshioka E, Yuasa M, Sata F, Saijo Y, et al. Cohort profile: the Hokkaido study on environment and children's health in Japan. Int J Epidemiol. 2011;40(3):611–8.
28. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet 1998;351(9111):1225–32.
29. Konishi H. A manual for the diagnoses of congenital anomalies (Gaihyou kikei sindan-no tebiki). Kanagawa, Japan: the study group on congenital anomalies in Kanagawa; 1981 (in Japanese).
30. Nishima S, Chisaka H, Fujiwara T, Furusho K, Hayashi S, Hiraba K, et al. Surveys on the prevalence of pediatric bronchial asthma in Japan: a comparison between the 1982, 1992, and 2002 surveys conducted in the same region using the same methodology. Allergol Int. 2009;58(1):37–53.

31. Okada E, Sasaki S, Saijo Y, Washino N, Miyashita C, Kobayashi S, et al. Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. Environ Res. 2012;112:118–25.
32. Yamada T, Morikawa M, Yamada T, Kishi R, Sengoku K, Endo T, et al. First-trimester serum folate levels and subsequent risk of abortion and preterm birth among Japanese women with singleton pregnancies. Arch Gynecol Obstet. 2013;287(1):9–14.
33. Iida T, Todaka T. Measurement of dioxins in human blood: improvement of analytical method. Ind Health. 2003;41(3): 197–204.
34. Todaka T, Hirakawa H, Kajiwara J, Hori T, Tobiishi K, Onozuka D, et al. Concentrations of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls in blood collected from 195 pregnant women in Sapporo City, Japan. Chemosphere. 2007;69(8):1228–37.
35. Todaka T, Hirakawa H, Kajiwara J, Onozuka D, Sasaki S, Miyashita C, et al. Concentrations of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls in blood and breast milk collected from pregnant women in Sapporo City, Japan. Chemosphere. 2011;85(11): 1694–700.
36. Todaka T, Hirakawa H, Tobiihi K, Iida T. New protocol of dioxins analysis in human blood. Fukuoka Igaku Zasshi. 2003;94(5):148–57.
37. Todaka T, Hori T, Hirakawa H, Kajiwara J, Yasutake D, Onozuka D, et al. Congener-specific analysis of non-dioxin-like polychlorinated biphenyls in blood collected from 195 pregnant women in Sapporo City, Japan. Chemosphere. 2008;73(6):923–31.
38. Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, et al. The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci. 2006;93(2): 223–41.
39. Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S, et al. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. Environ Health Perspect. 2004;112(11):1204–7.
40. Inoue K, Okada F, Ito R, Kawaguchi M, Okanouchi N, Nakazawa H. Determination of perfluorooctane sulfonate, perfluorooctanoate and perfluorooctane sulfonamide in human plasma by column-switching liquid chromatography-electrospray mass spectrometry coupled with solid-phase extraction. J Chromatogr B Anal Technol Biomed Life Sci. 2004;810(1):49–56.
41. Kashino I, Okada E, Matsuura H. Development of UPLC-MS/MS for the determination of eleven PFC in human plasma. In: Proceedings of Japan Society for Environmental Chemistry, Ehime, Japan, 11–13 July, 2012.
42. Kanazawa A, Miyasita C, Okada E, Kobayashi S, Washino N, Sasaki S, et al. Blood persistent organochlorine pesticides in pregnant women in relation to physical and environmental variables in The Hokkaido Study on Environment and Children's Health. Sci Total Environ. 2012;426:73–82.
43. Konishi K, Sasaki S, Kato S. Effect of prenatal exposure to dioxins on birth weight. In: Proceedings of Persistent Organic Pollutants (POPs) Research in Asia, 2008, pp 362–67.
44. Konishi K, Sasaki S, Kato S, Ban S, Washino N, Kajiwara J, et al. Effects of prenatal exposure to dioxins and methyl mercury on birth weight. Organohalogen Compd. 2007;69:2109–11.
45. Ito Y, Yokota H, Wang R, Yamanoshita O, Ichihara G, Wang H, et al. Species differences in the metabolism of di(2-ethylhexyl) phthalate (DEHP) in several organs of mice, rats, and marmosets. Arch Toxicol. 2005;79(3):147–54.
46. Tsuboi T, Kawai T, Araki A, Kishi R. Determination of human urinary metabolites of five phthalate by GC/MS—study of elementally school children in Sapporo, Japan. In: Proceedings of International Society of Exposure Science, Seattle, USA, 2012, p. 40.
47. Araki A, Kawai T, Tsuboi T, Ait Bamai Y, Takeda T, Yoshioka E, et al. Determination of phthalate metabolites in urine of children and their family—exposure assessment to plasticizer and flame retardants and their risk on children. In: Proceedings of International Society of Exposure Science, Seattle, USA, 2012, p. 72.
48. Yamamoto J, Hayama S, Sato N, Matsumura T, Kishi R, Sasaki S, et al. Analysis of bisphenol A in biological sample. In: Proceedings of 20th Symposium on Environmental Chemistry, Kumamoto, Japan, 2011, pp 610–11.
49. Sasaki S, Braimoh TS, Yila TA, Yoshioka E, Kishi R. Self-reported tobacco smoke exposure and plasma cotinine levels during pregnancy—a validation study in Northern Japan. Sci Total Environ. 2011;412–413:114–8.
50. Kashima H, Kato M, Handa T. Neuropsychological investigation of the frontal function in chronic schizophrenia: results of a modified Wisconsin card sorting test. Jpn J Clin Psychiatry. 1985;14:1479–89 (in Japanese).
51. Kashima H, Kato M. Wisconsin card sorting test (Keio Version) (KWCST). Brain Sci Ment Disord. 1995;6:209–16 (in Japanese).
52. Sasaki S. Nihongoban yoshokisei yakuwari kodo shakudo (J-PSAI) no sakusei: pre-School Activities Inventory no hoyaku. Stud Sociol Psychol Educ Inq Hum Soc. 2006;62:174–6.
53. Anme T, Shimada C, Katayama H. Evaluation of environmental stimulation for 18 months and the related factors. Nihon Koshu Eisei Zasshi. 1997;44(5):346–52.
54. Shiokawa H. Development of the life event questionnaire for parents: its use and reliability data. Jichi Med Univ J. 2007;30:165–72.
55. Kobayashi S, Sata F, Sasaki S, Ban S, Miyashita C, Okada E, et al. 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. Toxicol Lett. 2013;219(3): 269–78.
56. Sasaki S, Kondo T, Sata F, Saijo Y, Katoh S, Nakajima S, et al. Maternal smoking during pregnancy and genetic polymorphisms in the Ah receptor, CYP1A1 and GSTM1 affect infant birth size in Japanese subjects. Mol Hum Reprod. 2006;12(2):77–83.
57. Sasaki S, Sata F, Katoh S, Saijo Y, Nakajima S, Washino N, et al. Adverse birth outcomes associated with maternal smoking and polymorphisms in the N-Nitrosamine-metabolizing enzyme genes NQO1 and CYP2E1. Am J Epidemiol. 2008;167(6): 719–26.
58. Yila TA, Sasaki S, Miyashita C, Braimoh TS, Kashino I, Kobayashi S, et al. Effects of maternal 5,10-methylenetetrahydrofolate reductase C677T and A1298C Polymorphisms and tobacco smoking on infant birth weight in a Japanese population. J Epidemiol. 2012;22(2):91–102.
59. Konishi K, Sasaki S, Kato S, Ban S, Washino N, Kajiwara J, et al. Prenatal exposure to PCDDs/PCDFs and dioxin-like PCBs in relation to birth weight. Environ Res. 2009;109(7):906–13.
60. Nakajima S, Saijo Y, Kato S, Sasaki S, Uno A, Kanagami N, et al. Effects of prenatal exposure to polychlorinated biphenyls and dioxins on mental and motor development in Japanese children at 6 months of age. Environ Health Perspect. 2006;114(5): 773–8.
61. Washino N, Saijo Y, Konishi K. The effect of prenatal exposure to dioxins on cord serum IGE. In: Proceedings of the 27th International Symposium on Halogenated Persistent Organic Pollutants, Tokyo, Japan, 2–7 September, 2007
62. Miyashita C, Sasaki S, Saijo Y, Washino N, Okada E, Kobayashi S, et al. Effects of prenatal exposure to dioxin-like compounds on

allergies and infections during infancy. Environ Res. 2011;111(4):551–8.
63. Washino N, Saijo Y, Sasaki S, Kato S, Ban S, Konishi K, et al. Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environ Health Perspect. 2009;117(4):660–7.
64. Okada E, Kashino I, Matsuura H, Sasaki S, Miyashita C, Yamamoto J, et al. Temporal trends of perfluoroalkyl acids in plasma samples of pregnant women in Hokkaido, Japan, 2003–2011. Environ Int. 2013 (in press).
65. Okada E, Kashino I, Sasaki S, Miyashita C, Ikeno T, Araki A, et al. Relationship between prenatal exposure to perfluorinated compounds and allergic diseases in infants. In: Proceedings of International Society for Environmental Epidemiology, Basel, Switzerland, August, 2013
66. Rylander L, Stromberg U, Hagmar L. Dietary intake of fish contaminated with persistent organochlorine compounds in relation to low birthweight. Scand J Work Environ Health. 1996;22(4):260–6.
67. Baibergenova A, Kudyakov R, Zdeb M, Carpenter DO. Low birth weight and residential proximity to PCB-contaminated waste sites. Environ Health Perspect. 2003;111(10):1352–7.
68. Sonneborn D, Park HY, Petrik J, Kocan A, Palkovicova L, Trnovec T, et al. Prenatal polychlorinated biphenyl exposures in eastern Slovakia modify effects of social factors on birthweight. Paediatr Perinat Epidemiol. 2008;22(3):202–13.
69. Hertz-Picciotto I, Charles MJ, James RA, Keller JA, Willman E, Teplin S. In utero polychlorinated biphenyl exposures in relation to fetal and early childhood growth. Epidemiology. 2005;16(5):648–56.
70. Vartiainen T, Jaakkola JJ, Saarikoski S, Tuomisto J. Birth weight and sex of children and the correlation to the body burden of PCDDs/PCDFs and PCBs of the mother. Environ Health Perspect. 1998;106(2):61–6.
71. Guo YL, Lambert GH, Hsu CC, Hsu MM. Yucheng: health effects of prenatal exposure to polychlorinated biphenyls and dibenzofurans. Int Arch Occup Environ Health. 2004;77(3):153–8.
72. Masuda Y. Fate of PCDF/PCB congeners and change of clinical symptoms in patients with Yusho PCB poisoning for 30 years. Chemosphere. 2001;43(4–7):925–30.
73. Wang SL, Lin CY, Guo YLL, Lin LY, Chou WL, Chang LW. Infant exposure to polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls (PCDD/Fs, PCBs)—correlation between prenatal and postnatal exposure. Chemosphere. 2004;54(10):1459–73.
74. Suzuki G, Nakano M, Nakano S. Distribution of PCDDs/PCDFs and Co-PCBs in human maternal blood, cord blood, placenta, milk, and adipose tissue: dioxins showing high toxic equivalency factor accumulate in the placenta. Biosci Biotechnol Biochem. 2005;69(10):1836–47.
75. Fletcher T, Steenland K, Savitz D. 2009. Status Report: PFOA and Immune Biomarkers in Adults Exposed to PFOA in Drinking Water in the Mid Ohio Valley: [http://www.c8sciencepanel.org/pdfs/Status_Report_C8_and_Immune_markers_March2009.pdf] (Accessed 27 Sept 2011).
76. Harada K, Koizumi A, Saito N, Inoue K, Yoshinaga T, Date C, et al. Historical and geographical aspects of the increasing perfluorooctanoate and perfluorooctane sulfonate contamination in human serum in Japan. Chemosphere. 2007;66(2):293–301.
77. Jensen AA, Leffers H. Emerging endocrine disrupters: perfluoroalkylated substances. Int J Androl. 2008;31(2):161–9.
78. Kannan K, Corsolini S, Falandysz J, Fillmann G, Kumar KS, Loganathan BG, et al. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. Environ Sci Technol. 2004;38(17):4489–95.
79. Yoshioka W, Peterson RE, Tohyama C. Molecular targets that link dioxin exposure to toxicity phenotypes. J Steroid Biochem Mol Biol. 2011;127(1–2):96–101.
80. Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Lett. 2005;227(2):115–24.
81. Sørensen M, Autrup H, Møller P, Hertel O, Jensen SS, Vinzents P, et al. Linking exposure to environmental pollutants with biological effects. Mutat Res Rev Mutat Res. 2003;544(2–3):255–71.
82. Braimoh TS. Effects of maternal secondhand smoke exposure and gene polymorphisms of CYP1A1, EPHX1 and NAT2 on infant birth size. Doctoral thesis. Japan: Hokkaido University; 2012.
83. Damstra T, Barlow S, Bergman A, Kavlock R, Kraak Glen V. Global assessment of the state-of the science of endocrine disruptors. Geneva: World Health Organization; 2002.
84. Aschim EL, Nordenskjold A, Giwercman A, Lundin KB, Ruhayel Y, Haugen TB, et al. Linkage between cryptorchidism, hypospadias, and GGN repeat length in the androgen receptor gene. J Clin Endocrinol Metab. 2004;89(10):5105–9.
85. Thai HT, Kalbasi M, Lagerstedt K, Frisen L, Kockum I, Nordenskjold A. The valine allele of the V89L polymorphism in the 5-alpha-reductase gene confers a reduced risk for hypospadias. J Clin Endocrinol Metab. 2005;90(12):6695–8.
86. Radpour R, Rezaee M, Tavasoly A, Solati S, Saleki A. Association of long polyglycine tracts (GGN repeats) in exon 1 of the androgen receptor gene with cryptorchidism and penile hypospadias in Iranian patients. J Androl. 2007;28(1):164–9.
87. Lichtenstein P, Carlstrom E, Rastam M, Gillberg C, Anckarsater H. The genetics of autism spectrum disorders and related neuropsychiatric disorders in childhood. Am J Psychiatry. 2010;167(11):1357–63.
88. Ikeno T, Kobayashi S, Baba T, Kishi R. Literature review of the prevalence of attention-deficit hyperactivity disorder (ADHD) and its relation to environmental factors (ADHD no yuubyouritsu to youiku kankyou ni kansuru bunken review). Hokkaido J Public Health. 2011;25(2):53–9 (in Japanese).
89. Strang-Karlsson S, Raikkonen K, Pesonen AK, Kajantie E, Paavonen EJ, Lahti J, et al. Very low birth weight and behavioral symptoms of attention deficit hyperactivity disorder in young adulthood: The Helsinki study of very-low-birth-weight adults. Am J Psychiatry. 2008;165(10):1345–53.
90. Wolke D, Samara M, Bracewell M, Marlow N, Group EPS. Specific language difficulties and school achievement in children born at 25 weeks of gestation or less. J Pediatr. 2008;152(2):256–62.
91. Hille ETM, den Ouden AL, Saigal S, Wolke D, Lambert M, Whitaker A, et al. Behavioural problems in children who weigh 1000 g or less at birth in four countries. Lancet. 2001;357(9269):1641–3.
92. Hack M, Youngstrom EA, Cartar L, Schluchter M, Taylor HG, Flannery D, et al. Behavioral outcomes and evidence of psychopathology among very low birth weight infants at age 20 years. Pediatrics. 2004;114(4):932–40.
93. Hackett JA, Surani MA. DNA methylation dynamics during the mammalian life cycle. Philos Trans R Soc Lond B Biol Sci. 2013;368(1609):20110328.
94. Azumi K, Kobayashi S, Kishi R. Review: epigenetic research for the elucidation of the transgenelational effects of the environmental chemicals (Kankyou-kagaku bussitu no jisedaieikyou no kaimei ni okeru epigenetics kenkyuu). Hokkaido J Public Health. 2012;26(2):26–38 (in Japanese).
95. Baccarelli A, Bollati V. Epigenetics and environmental chemicals. Curr Opin Pediatr. 2009;21(2):243–51.

96. Bollati V, Baccarelli A. Environmental epigenetics. Heredity (Edinb). 2010;105(1):105–12.
97. Murphy SK, Adigun A, Huang Z, Overcash F, Wang F, Jirtle RL, et al. Gender-specific methylation differences in relation to prenatal exposure to cigarette smoke. Gene. 2012;494(1):36–43.
98. Hoyo C, Fortner K, Murtha AP, Schildkraut JM, Soubry A, Demark-Wahnefried W, et al. Association of cord blood methylation fractions at imprinted insulin-like growth factor 2 (IGF2), plasma IGF2, and birth weight. Cancer Causes Control. 2012;23(4):635–45.
99. Vrijheid M, Casas M, Bergstrom A, Carmichael A, Cordier S, Eggesbo M, et al. European birth cohorts for environmental health research. Environ Health Perspect. 2012;120(1):29–37.

Toxicology Letters 219 (2013) 269–278

Contents lists available at SciVerse ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

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

Sumitaka Kobayashi[a], Fumihiro Sata[b], Seiko Sasaki[a], Susumu Ban[c], Chihiro Miyashita[d], Emiko Okada[a], Mariko Limpar[a], Eiji Yoshioka[e], Jumboku Kajiwara[f], Takashi Todaka[g], Yasuaki Saijo[e], Reiko Kishi[d,*]

[a] *Department of Public Health Sciences, Hokkaido University Graduate School of Medicine, North 15, West 7, Kita-ku, Sapporo 060-8638, Hokkaido, Japan*
[b] *Department of Environmental Health, National Institute of Public Health, 2-3-6 Minami, Wako 351-0197, Saitama, Japan*
[c] *Faculty of Pharmaceutical Sciences, Suzuka University of Medical Science, 3500-3, Minami-Tamagaki-cho, Suzuka 513-8670, Mie, Japan*
[d] *Center for Environmental and Health Sciences, Hokkaido University, North 12, West 7, Kita-ku, Sapporo 060-0812, Hokkaido, Japan*
[e] *Department of Health Sciences, Asahikawa Medical University, Midorigaoka-Higashi 2-1-1-1, Asahikawa 078-8510, Hokkaido, Japan*
[f] *Fukuoka Institute of Health and Environmental Sciences, Mukaizano 39, Dazaifu 818-0135, Fukuoka, Japan*
[g] *Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Fukuoka, Japan*

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).

ARTICLE INFO

Article history:
Received 23 November 2012
Received in revised form 12 March 2013
Accepted 15 March 2013
Available online 23 March 2013

Keywords:
Aromatic hydrocarbon receptor
Cytochrome P450
Single-nucleotide polymorphism
Dioxin
Blood

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.

© 2013 Elsevier Ireland Ltd. All rights reserved.

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.

* Corresponding author at: Center for Environmental and Health Sciences, Hokkaido University, North 12, West 7, Kita-ku, Sapporo 060-0812, Hokkaido, Japan.
Tel.: +81 11 706 4746; fax: +81 11 706 4725.
E-mail address: rkishi@med.hokudai.ac.jp (R. Kishi).

0378-4274/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2013.03.013

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).

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 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).