Associations among maternal perfluoroalkyl substance levels, fetal sex-hormone enzymatic gene polymorphisms, and fetal sex hormone levels in the Hokkaido study

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Highlights

- Maternal perfluorooctanesulfonate levels associate with three sex hormone levels.
- There are changes in progesterone, dehydroepiandrosterone, and estradiol levels.
- Gene-perfluorooctanesulfonate interaction associates with sex hormone levels.
- Only female infants show gene-perfluorooctanesulfonate interaction.
- *Cytochrome P450 17A1* (rs743572) affects androstenedione and testosterone levels.

Abstract

Prenatal sex hormones affect fetal growth; for example, prenatal exposure to low levels of androgen accelerates female puberty onset. We assessed the association of perfluoroalkyl substances (PFASs) in maternal sera and infant genotypes of genes encoding enzymes involved in sex steroid hormone biosynthesis on cord sera sex hormone levels in a prospective birth cohort study of healthy pregnant Japanese women (n = 224) recruited in Sapporo between July 2002 and October 2005. We analyzed PFAS and five sex hormone levels using liquid chromatography-tandem mass spectrometry. *Cytochrome P450* (*CYP*) *17A1* (*CYP17A1* rs743572), *19A1* (*CYP19A1* rs10046, rs700519, and rs727479), *3β-hydroxysteroid dehydrogenase type 1* (*HSD3B1* rs6203), *type 2* (*HSD3B2* rs1819698, rs2854964, and rs4659175), *17β-hydroxysteroid dehydrogenase type 1* (*HSD17B1* rs605059, rs676387, and rs2676531), and *type 3* (*HSD17B3* rs4743709) were analyzed using real-time PCR. Multiple linear regression models were used to establish the influence of $\log_{10}$-transformed PFAS levels and infant genotypes on $\log_{10}$-transformed sex steroid hormone levels. When the interaction between perfluorooctanesulfonate (PFOS) levels and female infant genotype *CYP17A1*

(rs743572) on the androstenedione (A-dione) levels was considered, the estimated changes (95% confidence intervals) in A-dione levels against PFOS levels, female infant genotype *CYP17A1* (rs743572)-AG/GG, and interaction between them showed a mean increase of 0.445 (0.102, 0.787), mean increase of 0.392 (0.084, 0.707), and mean reduction of 0.579 (0.161, 0.997) (P_{int} = 0.007), respectively. Moreover, a female-specific interaction with testosterone levels was observed. A-dione and T levels showed positive main effects and negative interaction with PFOS levels and the female infant *CYP17A1* genotype.

Keywords

Perfluoroalkyl substances; Genotype; Cytochrome P450 17A1; Sex hormone; Gene-environment interaction; Sex difference

Abbreviations

A-dione: Androstenedione; CI: Confidence interval; CYP: Cytochrome P450; CYP17A1: Cytochrome P450 17A1; CYP19A1: Cytochrome P450 19A1; DHEA: Dehydroepiandrosterone; E_2: Estradiol; HSD3B: 3β-hydroxysteroid dehydrogenase; HSD3B1: 3β-hydroxysteroid dehydrogenase type 1; HSD3B2: 3β-hydroxysteroid dehydrogenase type 2; HSD17B: 17β-hydroxysteroid dehydrogenase; HSD17B1: 17β-hydroxysteroid dehydrogenase type 1; HSD17B3: 17β-hydroxysteroid dehydrogenase type 3; HWE: Hardy-Weinberg equilibrium; IQR: Inter-quartile range; LC/MS/MS: Liquid chromatography-tandem mass spectrometry; LD: Linkage disequilibrium; LOD: Limit of detection; LOQ: Limits of quantification; p_{int}: *p* for interaction; PFAS: Perfluoroalkyl substance; PFOA: Perfluorooctanoic acid; PFOS: Perfluorooctanesulfonate; P_4: Progesterone; SD: Standard deviation; SNP: Single nucleotide polymorphism; T: Testosterone

1. Introduction

Perfluoroalkyl substances (PFASs), such as perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), are poorly biodegradable compounds that persist and accumulate in the environment. Increased PFOS levels during pregnancy are associated with decreased dehydroepiandrosterone (DHEA) [1] and progesterone (P_4) [2] and increased estradiol (E_2) [2] levels in cord blood. They are associated with increased testosterone (T) levels in offspring at 15 years [3], but not associated with T and DHEA levels in offspring up to 20 years [4]. Increased PFOA levels were associated with increased DHEA levels in cord blood [1], luteinizing hormone and follicle-stimulating hormone levels in offspring at 15 years [5], but they were not associated with T and DHEA levels in female offspring up to 20 years [4]. Our previous study showed that increased PFOS, but not PFOA, levels are associated with decreased birth weight [6]. Thus, it is important to evaluate the effects of exposure to environmental chemicals on hormones as multiple hormones affect biological functions, and maintaining their balance is important to health.

The human biosynthetic pathway of sex steroid hormones related to PFAS exposure during pregnancy is shown in Fig. 1. Humans synthesize the male hormone T and the female hormone E_2 from cholesterol via P_4, DHEA, and androstenedione (A-dione) [7]. The cytochrome P450 (CYP) 17A1 (*CYP17A1*) gene encodes the CYP17 enzyme that is associated with the early stages of sex steroid hormone biosynthesis and catalyzes the conversion of pregnenolone and P_4 to DHEA and A-dione, respectively [7]. *CYP19A1* encodes the CYP19 enzyme (aromatase), which is associated with the final stage of sex steroid hormone biosynthesis and catalyzes the conversion of androgenic steroids, particularly A-dione to estrone and T to E_2 [7]. The 3β-hydroxysteroid dehydrogenase (HSD3B) type 1 (*HSD3B1*) and type 2 (*HSD3B2*) genes encode the HSD3B enzyme that is associated with the early stages of sex steroid hormone biosynthesis and catalyzes the conversion of pregnenolone to P_4 and DHEA to A-dione [7], whereas the 17β-hydroxysteroid dehydrogenase (HSD17B) type 1 (*HSD17B1*) and type 3 (*HSD17B3*) genes encode the HSD17B enzyme, which is associated with the final stages of sex steroid hormone biosynthesis and

catalyzes the conversion of A-dione to T and estrone to E_2 [7].

The genes regulating the biosynthesis of sex steroid hormones can affect the circulating sex hormone levels [8,9] and may modulate the susceptibility to PFASs during fetal growth and development. Previous studies have indicated that CYP directly metabolizes PFASs from the PFASs precursors [10-12]. PFOS increases *CYP19* and *HSD3B2* transcription levels [13] and *CYP17A1*, *HSD3B*, and *HSD17B* mRNA levels [14,15]. PFOA increases *CYP19* and *HSD3B2* transcription levels [13]. Therefore, PFASs could directly affect the expression of these enzymes. In this study, we focused on 12 single nucleotide polymorphisms (SNPs), which are well-known disease-susceptibility genes encoding enzymes related to sex steroid hormone biosynthesis. The SNPs of *CYP17A1* (A > G, dbSNP ID: rs743572) [16,17], *CYP19A1* (C > T; dbSNP ID: rs10046) [18], *CYP19A1* (C > T; dbSNP ID: rs700519) [19,20], *CYP19A1* (A > C; dbSNP ID: rs727479) [21], *HSD3B1* (T > C, Leu338Leu; rs6203) [22], *HSD17B1* (A > G, dbSNP ID: rs605059) [23], *HSD17B1* (C > A, dbSNP ID: rs676387) [24], and *HSD17B3* (T > C, dbSNP ID: rs4743709) [20,25] have been identified as genetic variants in genes associated with disease susceptibility. In addition, *HSD3B2* (C > T, rs1819698) [26], *HSD3B2* (A > T, rs2854964) [26], and *HSD17B1* (C > T, rs2676531) [27] have been linked to hypertensive disorder and familial amyloid polyneuropathy. *HSD3B2* (C > T, rs4659175) has been associated with salivary T levels in girls with transferrin levels of < 0.50 ng/dL according to a random forest analysis of genotypes with ± 5 kb of genes participating in T synthesis, transport, signaling, and metabolism [28]. However, it is unclear whether these genotypes in the fetus universally modify the associations between the PFAS levels during pregnancy and the sex steroid hormone levels in cord blood. Fetal genetic factors may be partially responsible for modifying the association between the PFAS and sex hormone levels.

Haplotypes, which refer to groups of genetic variants that tend to be inherited together, can be used not only to track inheritance patterns but also for keeping the statistical power of a study. Functional and informative structural gene patterns have been reported via therapeutic outcomes and evolutionary analysis [29,30]. Haplotype surveys suggest that it is valuable to interpret

unknown or known functional genotypes in their functional, structural gene patterns, which may help resolve the causal relationship regarding gene-environment interactions found across a wide variety of epidemiological studies. In a specific population, a specific region on the genome with almost no trace of genetic recombination is a haplotype block, and the linkage disequilibrium (LD) of this block is very strong. Even if you do not know the haplotype frequency, you can examine the haplotype block using LD analysis. A tag SNP refers to an SNP selected to identify a combination of haplotypes existing in the same compartment. In particular, the utilization of tag SNP by using the haplotype block instead of typing all the SNPs can improve the efficiency of statistical analysis.

Many previous studies have investigated the association between environmental exposures and sex hormones. However, the effect of genetic factors has not been investigated in these studies. It is thought that heredity and environment influence each other on the sex hormone levels. Therefore, we need to re-examine the relationship between environmental factors and sex hormones while considering the genetic factors. In our previous studies, we have investigated the association between maternal genotypes and prenatal chemical exposure due to various factors, such as smoking, consumption of caffeine, and exposure to dioxins and PFASs on fetal growth [31-35], and the association between the PFAS levels in the sera of pregnant Japanese women and the sex steroid hormone (P_4, DHEA, A-dione, T, and E_2) levels in cord blood [1,2]. We hypothesized that there might be specific genotypes of sex hormone biosynthesis genes and increased exposure to PFASs results in sex-specific differences in the levels of sex hormones. Following up on the studies conducted by Goudarzi et al. [1] and Itoh et al. [2], we used 12 SNP tags (*CYP17A1* rs743572; *CYP19A1* rs10046, rs700519, and rs727479; *HSD3B1* rs6203; *HSD3B2* rs1819698, rs2854964, and rs4659175; *HSD17B1* rs605059, rs676387, and rs2676531; and *HSD17B3* rs4743709) encoding enzymes that are involved in sex steroid hormone biosynthesis, and explored the association between PFAS levels during pregnancy in maternal blood, infant genotypes, and sex hormone (P_4, DHEA, A-dione, DHEA/A-dione, T, and E_2, T/E_2)

levels in cord blood.

2. Materials and methods

2.1. Study participants

This prospective birth cohort study was based on the Hokkaido Study on Environment and Children's Health (Sapporo cohort). The study protocol has been described previously [36-38]. Briefly, from July 2002 to October 2005, pregnant Japanese women (n = 514) were recruited from a local obstetrics and gynecology hospital in Sapporo City. Of these, 10 participants withdrew. Of the remaining 504, 224 participants provided complete data on PFOS and PFOA levels, infant genotypes, and sex hormone levels (Fig. 2).

2.2. Ethical approval

Written informed consent was obtained from all the participants. All the procedures were conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Institutional Ethical Board for Human Gene and Genome Studies and the Epidemiological Studies Programs of the Hokkaido University Center for Environmental and Health Sciences (approval number 119).

2.3. Data collection

Each participant completed a self-administered questionnaire at enrollment regarding maternal age, annual household income, maternal smoking during the third trimester, and maternal alcohol consumption during pregnancy. Medical records were also obtained to collect information on parity and infant sex.

2.4. Measurement of maternal serum PFOS and PFOA levels

PFOS and PFOA levels were measured in 447 maternal blood samples. Those of the other

participants were not analyzed because they were either not available or the sample volume was insufficient. Of the 447 blood samples, 228 were collected during pregnancy (mean ± standard deviation of gestational weeks at blood sample collection: 33.2 ± 3.7 weeks) and 159 were obtained after delivery owing to anemia during pregnancy. Hence, we analyzed the 447 samples for PFOS and PFOA levels using liquid chromatography-tandem mass spectrometry (LC/MS/MS), according to the methods described previously [6,39]. All the samples surpassed the limit of detection (LOD; 0.50 ng/mL) for PFOS. However, 16 (5.9%) samples had PFOA levels below the LOD (0.50 ng/mL), and these cases were assigned a value of 0.25 ng/mL (50% of LOD).

2.5. Assessment of infant genotype

We evaluated the genotypes of *CYP17A1* (rs743572), *CYP19A1* (rs10046, rs700519 and rs727479), *HSD3B2* (rs4659175), *HSD17B1* (rs2676531), and *HSD17B3* (rs4743709) in 261 participants and those of *HSD3B1* (rs6203), *HSD3B2* (rs1819698 and rs2854964), and *HSD17B1* (rs605059 and rs676387) in 297 participants. The remaining 217 (= 514－297) participants were excluded because they either had a cesarean birth, or their cord blood could not be collected because they were registered in the cord blood bank. Cord blood in the blood bank was not available for chemical analysis because the donors signed an agreement to allow its use for patients who need a transplant. Of the 297 participants, 36 were not available or lacked sufficient blood volume for the genetic analyses because of their use in previous studies. Cord blood samples were collected at birth, and 400 μL of each sample was used for genomic DNA extraction, isolation, and purification using a QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) and a Maxwell 16 DNA purification kit (Promega, Madison, WI, USA) according to the manufacturer's instructions [40]. Seven genotypes were evaluated, i.e., *CYP17A1* (rs743572), *CYP19A1* (rs10046, rs700519, and rs727479), *HSD3B2* (rs4659175), *HSD17B1* (rs2676531), and *HSD17B3* (rs4743709), using high-throughput pre-amplification gene expression (Supplementary Method 1) and real-time PCR on dynamic chips (Supplementary Method 2),

whereas these five genotypes, i.e., *HSD3B1* (rs6203), *HSD3B2* (rs1819698 and rs2854964), and *HSD17B1* (rs605059 and rs676387) were assessed using TaqMan gene expression measurements (Supplementary Method 3).

2.6. Measurement of cord serum sex hormone level

The sex hormone levels in the cord blood samples were measured in 294 participants. Other participants were excluded for the reasons stated in Section 2.5. The concentrations of P_4, DHEA, A-dione, T, and E_2 were determined using LC/MS/MS according to the methods described previously [1,2,41,42]. All measurements were performed by ASKA Pharma Medical Co. Ltd. (Kanagawa, Japan). Samples below the limits of quantification (LOQ) were assigned values that were 50% of their respective LOQs.

2.7. Statistical analyses

Of the 224 participants, two (0.9 %) had missing data on annual household income. Using simple imputation, the participants were assigned to the annual household income group of < 5 million Japanese yen (the most frequent group). First, the associations between the variables in males and females were analyzed using the independent *t*-test, Mann-Whitney *U*-test, and chi-squared test. The association between maternal gestational weeks at blood sample collection and PFOS, PFOA, and sex hormone levels were analyzed using Spearman's rank correlation coefficients. Second, a chi-squared test was employed to test whether the frequency of genotype distribution conformed to the Hardy-Weinberg equilibrium (HWE). Third, a case-control study was set up with a lower than median A-dione level (n_{case} = 109), and the linkage disequilibrium (LD) was evaluated using linkage analyses. Fourth, PFOS, PFOA, and sex hormone levels were $\log_{10}$-transformed before the following analyses because of their non-normal distribution. Multiple linear regression analyses were used to evaluate the association between PFOS or PFOA levels and sex hormone levels in both the crude and adjusted models. Maternal age,

maternal smoking during the third trimester, maternal alcohol consumption during pregnancy, infant sex (only total infant), maternal blood sampling periods, and infant birth weight were adjusted using the multiple linear regression analyses, except for in the crude models. Analyses stratified by infant sex were also conducted. Fifth, multiple linear regression analyses were used to evaluate the interaction between PFOS or PFOA levels and infant sex on sex hormone levels in both the crude and adjusted models. The covariates were the same as those used in the fourth analysis. Sixth, multiple linear regression analyses were used to evaluate the association between the infant genotypes and PFOS, between them and PFOA, or between them and sex hormone levels in both the crude and adjusted models. The covariates were the same as those used in the fourth analysis. Analyses stratified by infant sex were also conducted. Maternal sampling periods were excluded from the covariates only when the outcomes were PFOS or PFOA levels. Seventh, multiple linear regression analyses were used to evaluate the interaction between PFOS or PFOA levels and infant genotypes on sex hormone levels in both the crude and adjusted models. The covariates were the same as those used in the fourth analysis. Analyses stratified by infant sex were also conducted. In addition, multiple linear regression analyses were used to evaluate the association between PFOS levels and sex hormone levels in both the crude and adjusted models after stratification based on the infant genotype *CYP17A1* (rs743572) because only the effect of the interactions between PFOS levels and infant genotype *CYP17A1* (rs743572) on A-dione and T levels were statistically significant. The covariates were the same as those used in the fourth analysis. Analyses stratified by infant sex were also conducted.

The PFAS-sex interaction term was defined as "$\log_{10}$-transformed PFOS or PFOA levels (continuous) * sex (0 = males and 1 = females)" and PFAS-gene interaction term was defined as "$\log_{10}$-transformed PFOS or PFOA levels (continuous) * genotype (0 = referent genotype and 1 = genotype to be compared)". The interaction term was included in the multiple linear regression models except for the stratified analysis.

Furthermore, we refuted or confirmed the validity of the results in all participants, using the sensitivity analyses by restricting participants to those that maternal blood samples were collected during pregnancy (before delivery). Multiple linear regression analyses in the sensitivity analyses were set up using the same covariates in the multiple linear regression models in all participants.

Data were considered statistically significant at $p < 0.05$. The p-value for interaction was also considered significant if $p < 0.05$. All statistical analyses were performed using SPSS software version 26 (IBM Corp., Armonk, NY, USA), except for the linkage analyses. Linkage analyses were performed using Haploview 4.2 software (Broad Institute of Massachusetts Institute of Technology and Harvard, USA) [40].

3. Results

Characteristics of the study participants are shown in Table 1. Mean maternal age (standard deviation; SD) was 30.0 (4.8) years of age. Median PFOS and PFOA levels (inter-quartile range; IQR) in the maternal sera were 5.0 (3.3, 6.9) ng/mL and 1.4 (0.9, 2.0) ng/mL, respectively. Median (IQR) PFOS levels did not differ between the 224 participants (5.0 [3.3, 6.9] ng/mL) included in the study and the 223 participants (5.5 [3.6, 7.2] ng/mL; $p = 0.174$) excluded from the study. However, median (IQR) PFOA levels differed between the included participants (1.4 [0.9, 2.0] ng/mL) and the excluded participants (1.2 [0.8, 1.6] ng/mL; $p = 0.003$; Supplementary Table 1). The median P_4, DHEA, A-dione, T, and E_2 (IQR) in the cord sera were 217.8 (173.9, 282.4) ng/mL, 2.3 (1.8, 3.0) ng/mL, 0.45 (0.36, 0.57) ng/mL, 84.4 (59.5, 111.2) pg/mL, and 4.8 (3.3, 7.1) ng/mL, respectively. The male and female groups differed in terms of median DHEA (2.1 ng/mL vs. 2.4 ng/mL; $p = 0.002$) and T (92.9 pg/mL vs. 73.1 pg/mL; $p < 0.001$) levels in the cord sera. Correlations between PFOS levels and gestational age, between PFOA levels and gestational weeks at blood sample collection, and between sex

hormone levels and gestational weeks at blood sample collection were not statistically significant (Supplementary Table 2).

Infant genotype frequencies are summarized in Table 2. The distribution of all 12 SNPs in the 224 infants satisfied HWE (χ^2-test: all $p > 0.05$).

The LD plot for the 12 SNPs is shown in Fig. 3. The LD parameter (D') for *CYP19A1* (rs10046, rs700519, and rs727479) was 0.90-0.91. The D' values for *HSD3B2* (rs1819698, rs2854964, and rs4659175) and *HSD17B1* (rs605059, rs676387, and rs2676531) were 0.97, and 0.99, respectively. Except for these values, D' was < 0.90.

The effects of maternal PFOS and PFOA levels on infant sex hormone levels, stratified by infant sex, are summarized in Table 3. Multiple linear regression analysis showed that the PFOS levels were associated with lower P_4 (mean reduction = 0.400 [95% confidence interval (CI): 0.201, 0.598]) and T/E_2 (mean reduction = 0.159 [95% CI: 0.005, 0.313]) levels, and higher DHEA (mean increase = 0.359 [95% CI: 0.167, 0.552]), DHEA/A-dione (mean increase = 0.338 [95% CI: 0.110, 0.565]), and E_2 (mean increase = 0.166 [95% CI: 0.005, 0.326]) after adjustment for the covariates in all infants. This implies that the PFOS levels were associated with lower P_4 (mean reduction = 0.270 [95% CI: 0.029, 0.512]) and T/E_2 (mean reduction = 0.305 [95% CI: 0.112, 0.497]) levels, and higher DHEA (mean increase = 0.249 [95% CI: 0.015, 0.483]) levels after adjusting for covariates in male infants. This also implies that the PFOS levels were associated with lower P_4 (mean reduction = 0.517 [95% CI: 0.205, 0.829]) and higher DHEA (mean increase = 0.448 [95% CI: 0.146, 0.751]) levels after adjusting for the covariates in female infants. We further performed a sensitivity analysis, the results of which showed that the 95% confidence interval tended to be wider but did not change the original results (Supplementary Table 3). Interestingly, for the T/E_2 levels, we observed a statistically significant interaction between PFOS levels and infant sex only after adjusting for the covariates (p for interaction [p_{int}] = 0.045; Supplementary Table 4). In addition, there was no association

between PFOA levels and sex hormone levels after adjustment for covariates. The results were confirmed using sensitivity analysis (Supplementary Table 5).

The combined associations of PFOS levels and infant genotype *CYP17A1* (rs743572) on sex hormone levels in female infants are presented in Table 4 (see also Fig. 4A and 4B). Multiple linear regression analysis showed a significant association between the interaction between PFOS levels and infant genotype *CYP17A1* (rs743572) on the A-dione and T levels after adjustment for the covariates in female infants. When the interaction between PFOS levels and infant genotype *CYP17A1* (rs743572) on the A-dione levels was considered after adjustment for the covariates, the estimated changes (95% CI) in the A-dione levels per one unit increase in PFOS levels had a mean increase of 0.445 (0.102, 0.787), those in the A-dione levels of infant genotype *CYP17A1* (rs743572)-AG/GG compared to AA had a mean increase of 0.396 (0.084, 0.707), and those in the A-dione levels of the interaction term between PFOS and *CYP17A1* (rs743572)-AG/GG had a mean reduction of 0.579 (0.161, 0.997) (p_{int} = 0.007). When the interaction between PFOS levels and infant genotype *CYP17A1* (rs743572) on the T levels was considered after adjustment for the covariates, the estimated changes (95% CI) in the T levels per one unit increase in PFOS levels had a mean increase of 0.641 (0.191, 1.091), those in the T levels of infant genotype *CYP17A1* (rs743572)-AG/GG compared to AA had a mean increase of 0.595 (0.186, 1.003), and those in the A-dione levels of the interaction term between PFOS and *CYP17A1* (rs743572)-AG/GG had a mean reduction of 0.856 (0.307, 1.404) (p_{int} = 0.003). The results were confirmed using sensitivity analysis (Supplementary Table 6). There was no statistically significant interaction between PFOS levels and 11 SNPs of *CYP19A1*, *HSD3B1*, *HSD3B2*, *HSD17B1*, and *HSD17B3* on sex hormone levels (data not shown). In addition, there was no statistically significant interaction between PFOA levels and any of the 12 SNPs on sex hormone levels (data not shown).

The effects of maternal PFOS levels on infant sex hormone levels stratified by the female infant genotype *CYP17A1* (rs743572) are shown in Table 5. The estimated changes in A-dione

levels per one-unit increase of PFOS levels (95% CI) were a mean increase of 0.494 (0.059, 0.930) in female infants with the AA genotype. The estimated changes in T levels per one-unit increase of PFOS levels (95% CI) were a mean increase of 0.679 (0.176, 1.187) in female infants with the AA genotype. However, there were not significant change in A-dione and T levels in female infants with the AG/GG genotype. The results were confirmed using sensitivity analysis (Supplementary Table 7).

4. Discussion

In this study, we observed that the interaction between the prenatal PFOS levels and the infant genotype *CYP17A1* (rs743572) influenced the A-dione and T levels in all participants and female infants. Therefore, these gene-environment interactions exhibit sex differences.

To the best of our knowledge, only five studies have explored the association between prenatal exposure to PFASs and sex hormone levels [1-5]. In our previous studies, we have revealed that an increase in prenatal PFOS exposure (median: 5.2 ng/mL) was associated with increased cord DHEA levels in all participants and cord E_2 levels in male infants and decreased cord P_4 levels in all participants. An increase in prenatal PFOA exposure (median: 1.4 ng/mL) was associated with decreased cord DHEA levels [1,2]. Contrary to our results and previous reports, Kristensen et al. [4] observed that increased DHEA levels were not associated with higher PFOS (median: 21.1 ng/mL) and PFOA (median: 3.6 ng/mL) exposure. This is the first study to investigate sex differences in the association between prenatal PFAS levels and fetal sex hormone levels.

Two studies have evaluated the influence of prenatal exposure to PFOS and PFOA on hormone levels among 15-year-old girls (median: 19.2 ng/mL for PFOS; 3.6 ng/mL for PFOA) [3] and adult men (median: 21.2 ng/mL for PFOS; 3.8 ng/mL for PFOA) [5]. They found that increased prenatal PFOS and PFOA exposures were associated with increased T levels in 15-year-old girls, but no change in T and E_2 levels was found in adult men [3,5]. In this study,

increased PFOS levels (median: 5.4 ng/mL), but not PFOA levels (median:1.4 ng/mL), were associated with P_4, DHEA, DHEA/A-dione, E_2, and T/E_2 levels. Therefore, these results are in partial accordance with those of the present study. Differences in the PFOA exposure IQR range (0.9-2.1 ng/mL in this study vs. 2.7-4.7 ng/mL reported by Maisonet et al. [3] and Vested et al. [5]) could contribute to the discrepancies in the findings of the different studies.

The effects of the infant genotype *CYP17A1* (rs743572) on maternal PFOS and PFOA levels were not observed (Supplementary Table 8). The *CYP17A1* mRNA or protein expression levels of CYP17A1 were reduced in rodents administered with more than 5 or 10 mg/kg PFOS daily [44,45]. CYP17 catalyzes the conversion of P_4 to A-dione via an intermediate product [7]. Increased PFOS levels may decrease *CYP17A1* mRNA, CYP17A1 protein levels, the enzymatic activation of CYP17A1, and the production of A-dione and T, which are located downstream of A-dione.

The 5'-untranslated region of the *CYP17* gene contains *CYP17A1* (A>G, rs743572) [46]. It locates an Sp1-type (CCACC box) promoter site 34-base pair upstream of the initiation site of translation [46]. This base pair change creates a CCACC box. It is supposed that the number of 5' promoter elements correlates with the promoter activity [47]. Increased PFOS levels may affect the 5'-untranslated regions of *CYP17*, cause a decrease in the promoter activity of its gene, and then decrease the enzymatic CYP17A1 activation.

To date, there are no reports on the association between *CYP17A1* (rs743572) and promoter activity. In previous epidemiological studies, postmenopausal women with the *CYP17A1* GG genotype had higher levels of estrone compared to those with the *CYP17A1* AA genotype [48] and postmenopausal women with the *CYP17A1* AG or GG genotype had higher levels of E_2 [49,50]. E_2 is located downstream of A-dione and T [7]. Previous studies have shown that enzymatic CYP17A1 activation may be higher in the infant genotype *CYP17A1* (rs743572) AG/GG than in AA.

A-dione and T levels in the cord sera were not influenced by the infant genotype *CYP17A1* (rs743572) and PFOS levels in maternal blood (Supplementary Table 8). The *CYP17A1* (rs743572) genotype was not associated with DHEA or A-dione levels in four previous studies [8,48,51,52]. The results of these previous studies are similar to those of the present study.

Cord sera T levels, but not cord sera A-dione levels, were influenced by infant sex. Interaction analysis did not reveal any interaction between maternal PFOS levels and infant sex on infant A-dione and T levels. This result suggests that no interaction exist between infant sex and maternal sera PFOS levels during pregnancy in A-dione and T levels in the cord sera.

We found an interaction between maternal sera PFOS levels and the infant genotype *CYP17A1* (rs743572) on infant A-dione and T levels. For this genotype, there was an obvious positive correlation with A-dione and T levels in the AA genotype, but no obvious negative correlation with A-dione and T levels in the AG/GG genotype. This result implies that interactions might exist between the infant genotype *CYP17A1* (rs743572) and maternal sera PFOS levels during pregnancy in A-dione and T levels in the cord sera.

In female infants, the regression coefficient of PFOS and the *CYP17A1* genotype on A-dione or T levels showed a positive main effect but showed a negative interaction term (Table 4). This indicates that the effect of PFOS on A-dione or T becomes positive or negative depending on the *CYP17A1* genotype. In the AA genotype, there was a significant association between PFOS and A-dione or T, and the positive change in A-dione or T levels by PFOS was small (Table 5). In the AG/GG genotype, the negative change in A-dione or T levels by PFOS was not significant (Table 5). It can be interpreted that the relationship between PFOS and A-dione or T levels changes significantly in the negative direction when changing from AA to AG/GG genotype (Table 5 and Fig. 4A and 4B). We speculated that the *CYP17A1* genotype canceled the negative interaction term shown in the effects. Therefore, it was considered that AA genotype might be a genetically susceptible population that is easily affected by A-dione or

T levels by the increase in PFOS levels, suggesting a possible biological mechanism underlying this finding.

Many studies have determined the genetic susceptibility of genotypes *CYP19A1* (rs10046, rs700519, and rs727479), *HSD3B1* (rs6203), *HSD3B2* (rs1819698 and rs2854964), *HSD17B1* (rs605059, rs676387, and rs2676531), and *HSD17B3* (rs4743709) [18,19,21-25,27]. However, these genotypes did not modify the association between PFOS and sex hormone levels in our study. In this study, the maternal PFOS levels in the blood sera were low, and approximately 30% of PFOS was transferred to the fetus from the mother through the placenta [39]; therefore, their genotypes and low PFOS levels in the fetuses might not be influenced by sex hormone levels.

One of the main strengths of this study is the accurate measurement of blood PFOS and PFOA levels using LC/MS/MS. Furthermore, we minimized bias using a prospective birth cohort study design. However, this study has several limitations. First, we measured cord sex hormone levels. Hormone levels changed from the end of gestation to after birth [53]. The association between sex hormone levels and gestational age is not significant in this study, and hence, the effects of individual hormonal variations (inter-individual variations) among the participants depending on the gestational age are limited. However, when interpreting our results, it should be noted that hormonal variations within an individual (intra-individual variations) change within short periods, and thus, hormonal variation might not be accurately reflected by a single measurement. Second, although the percentage of smokers and alcohol consumers in our cohort, that is the Hokkaido region, tended to be the highest among all the other regions in Japan [54], neither smoking nor alcohol consumption status affected our results (data not shown). Third, there are problems with multiple comparisons. Since it is a multiple comparison, the *p*-values of PFAS levels, genotype, and sex hormone levels must be corrected. When two or more SNPs are in strong LD, if one SNP can be known, information on the other SNPs can be determined (tagSNP). Even if tagSNP is used, the number of SNPs remains nine.

Thus, the number of patterns for the combinations of exposure, tagSNPs, and sex hormones was 90. For the Bonferroni correction, $p \leq 0.0005$ (= 0.05/90) was significant. When these corrections were applied, the results of this study were not significant. Finally, since the results have not been replicated in any external cohort thus far, the generalizability of the results is limited. We consider that the results of this study can be adapted for a similar population with comparable exposure, outcome, and genetic distribution.

5. Conclusion

For the first time, we showed that the infant genotype *CYP17A1* (rs743572) and sex differences play an important role in determining how maternal PFOS exposure during pregnancy influences fetal sex hormone levels.

Authors' contributions

S.K. contributed to the study design, data acquisition, analysis, interpretation, and manuscript drafting. F.S., A.A., C.M., S.I., and H.G. contributed substantially to data acquisition, analysis, and interpretation. Y.I. contributed to data analysis. T.M., K.M., N.S., and K.C. contributed to data acquisition. R.K. made substantial contributions to the conception of the study design, data acquisition, analysis, interpretation, and supervision. All authors critically revised the manuscript and approved the final version for publication.

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Conflicts of interest

The authors declare no conflicts of interest.

Data statement

The data and materials used to derive our conclusions are unsuitable for public deposition due to ethical restrictions and specific legal framework in Japan. It is prohibited by the Act on the Protection of Personal Information (Act No. 57 of May 30, 2003, amended on September 9, 2015) to publicly deposit data containing personal information. The Ethical Guidelines for Epidemiological Research enforced by the Japan Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare also restrict the open sharing of the epidemiologic data. All inquiries about access to data should be sent to rkishi@med.hokudai.ac.jp. The person responsible for handling inquiries sent to this e-mail address is Professor Reiko Kishi, Principal Investigator of the Hokkaido Study on Environment and Children's Health, Center for Environmental and Health Sciences, Hokkaido University.

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Figure Legends

Fig. 1. Simplified schematic pathway of sex steroid biosynthesis

Abbreviations: CYP17A1, cytochrome P450 17A1; CYP19A1, cytochrome P450 19A1; HSD3B1, 3β-hydroxysteroid dehydrogenase type 1; HSD3B2, 3β-hydroxysteroid dehydrogenase type 2; HSD17B1, 17β-hydroxysteroid dehydrogenase type 1, and HSD17B3, 17β-hydroxysteroid dehydrogenase type 3.

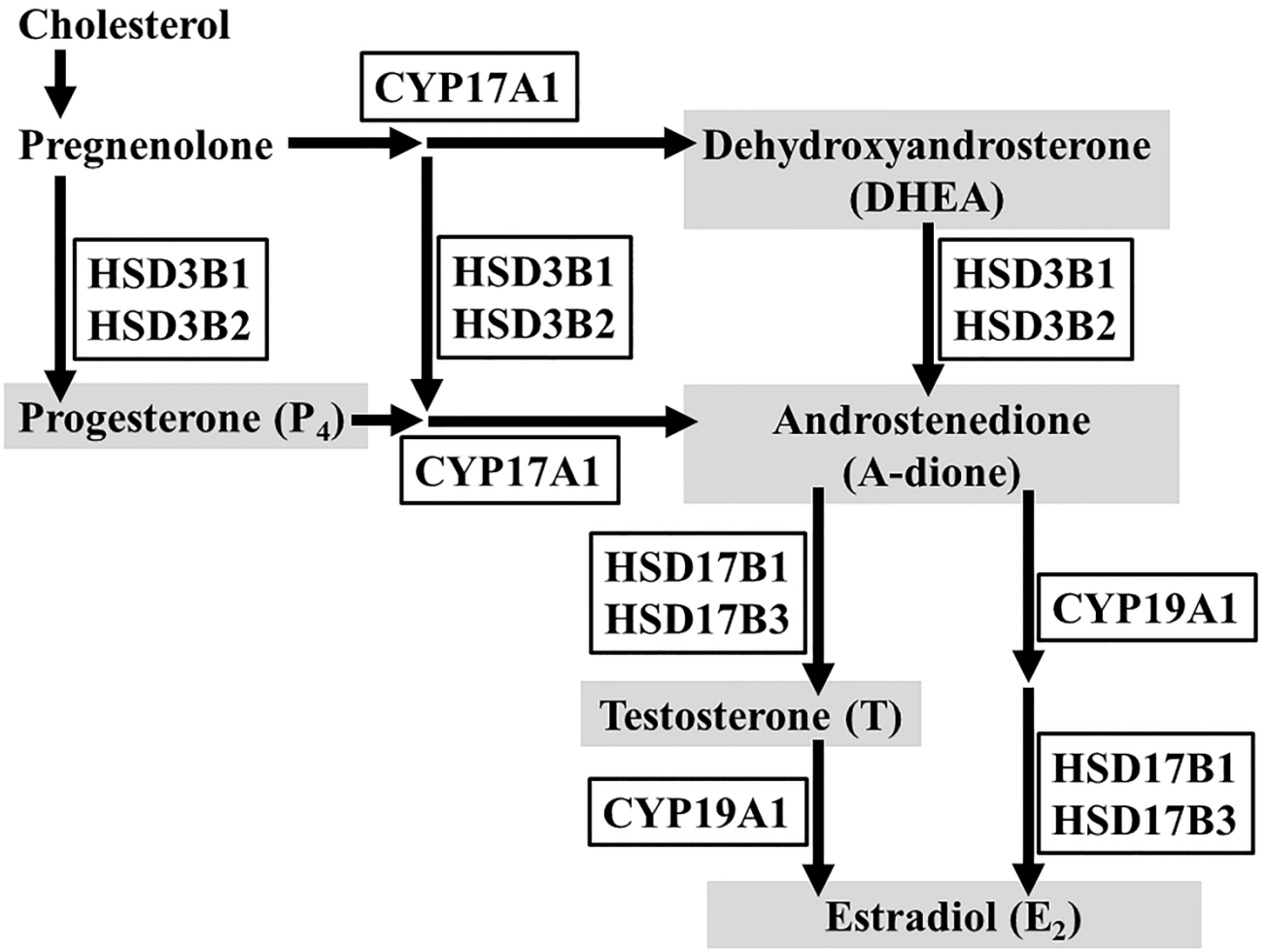
Fig. 2. Participant selection flow diagram

Fig. 3. Linkage disequilibrium (LD) plot for the *CYP17A1* (dbSNP ID: rs743572), *CYP19A1* (dbSNP ID: rs10046, rs700519, and rs727479), *HSD3B1* (dbSNP ID: rs6203), HSD3B2 (dbSNP ID: rs1819698, rs2854964, and rs4659175), *HSD17B1* (dbSNP ID: rs605059, rs676387, and rs2676531), and *HSD17B3* (dbSNP ID: rs4743709) single nucleotide polymorphisms (SNPs) in infants

The LD parameter (D') value is provided within the boxes ($-1 \leq D' \leq 1$).
A D' of 1 represents perfect genetic linkage.
A D' of 0.90, 0.91, 0.97, and 0.99 (as indicated 90, 91, 97, and 99 in this figure) represents approximately perfect genetic linkage.

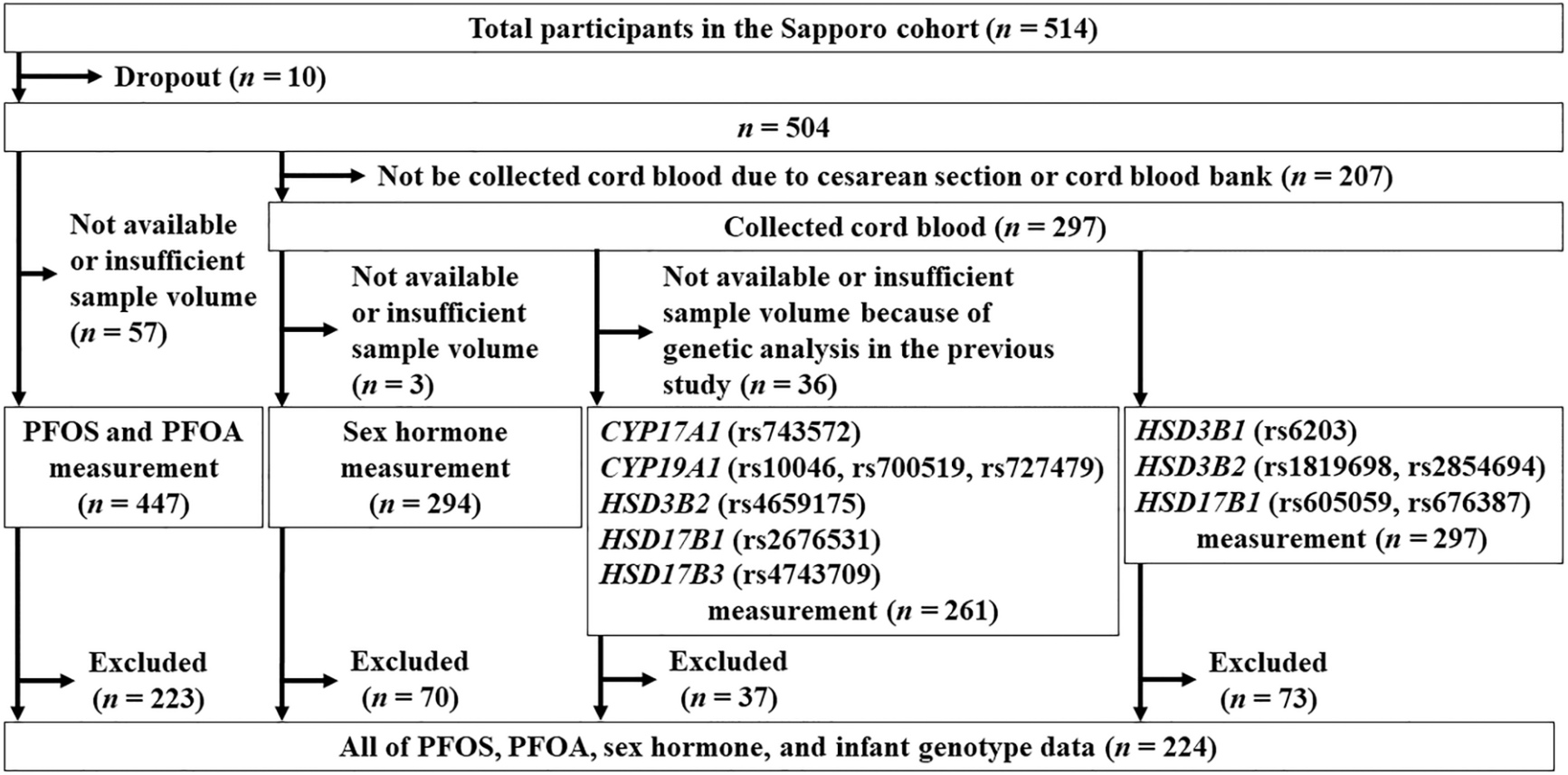
Fig. 4. Interaction plots of maternal PFOS levels during pregnancy and infant *CYP17A1* (rs743572) genotype on (A) A-dione or (B) T levels in cord blood in female infants

1 Fig. 1


Cholesterol
CYP17A1
Pregnenolone
Dehydroxyandrosterone (DHEA)
HSD3B1 HSD3B2
HSD3B1 HSD3B2
HSD3B1 HSD3B2
Progesterone (P_4)
CYP17A1
Androstenedione (A-dione)
HSD17B1 HSD17B3
CYP19A1
Testosterone (T)
CYP19A1
HSD17B1 HSD17B3
Estradiol (E_2)

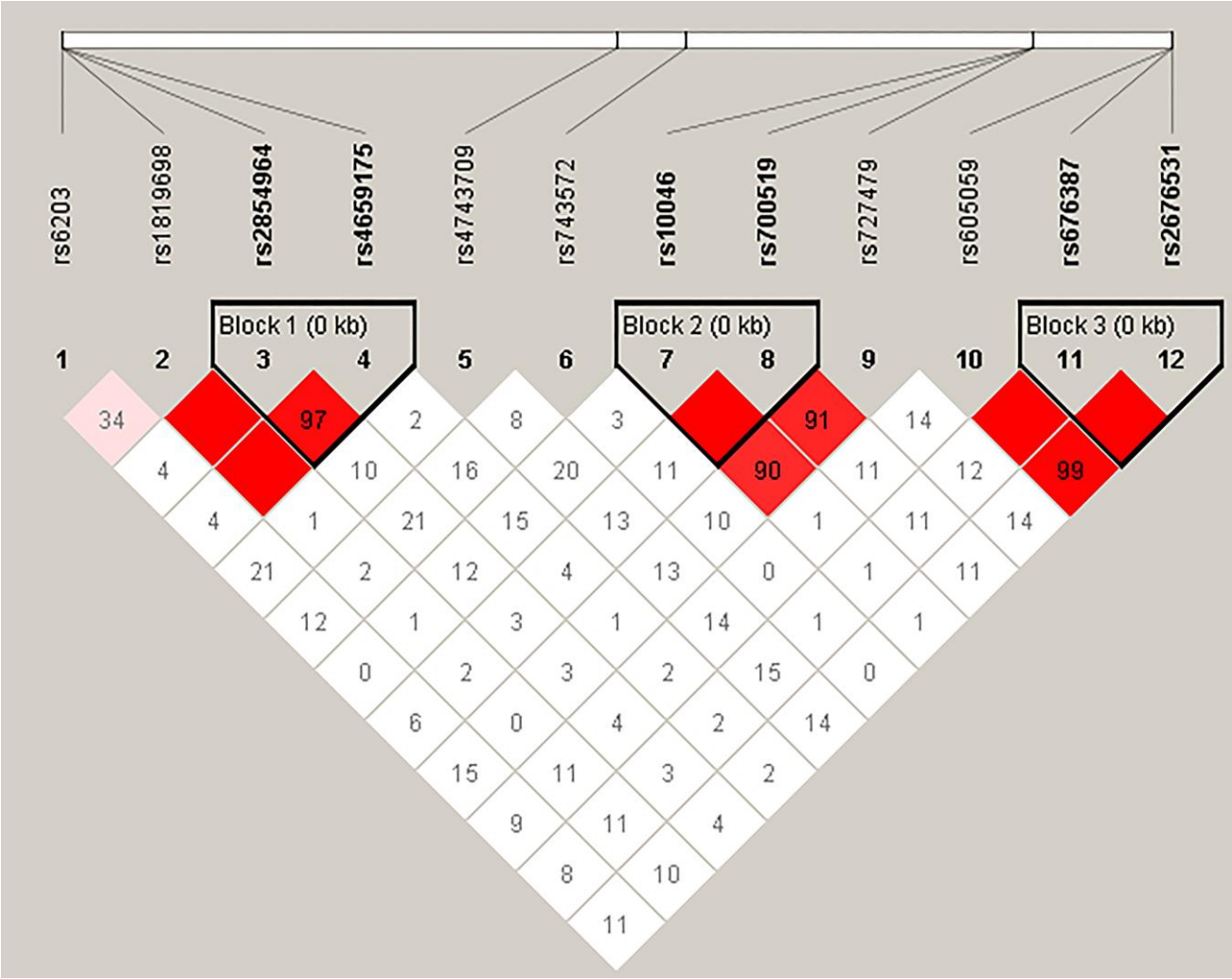
2

1 Fig. 2


Total participants in the Sapporo cohort (n = 514)
Dropout (n = 10)
n = 504
Not be collected cord blood due to cesarean section or cord blood bank (n = 207)
Collected cord blood (n = 297)
Not available or insufficient sample volume (n = 57)
Not available or insufficient sample volume (n = 3)
Not available or insufficient sample volume because of genetic analysis in the previous study (n = 36)
PFOS and PFOA measurement (n = 447)
Sex hormone measurement (n = 294)
CYP17A1 (rs743572)
CYP19A1 (rs10046, rs700519, rs727479)
HSD3B2 (rs4659175)
HSD17B1 (rs2676531)
HSD17B3 (rs4743709)
measurement (n = 261)
HSD3B1 (rs6203)
HSD3B2 (rs1819698, rs2854694)
HSD17B1 (rs605059, rs676387)
measurement (n = 297)
Excluded (n = 223)
Excluded (n = 70)
Excluded (n = 37)
Excluded (n = 73)
All of PFOS, PFOA, sex hormone, and infant genotype data (n = 224)

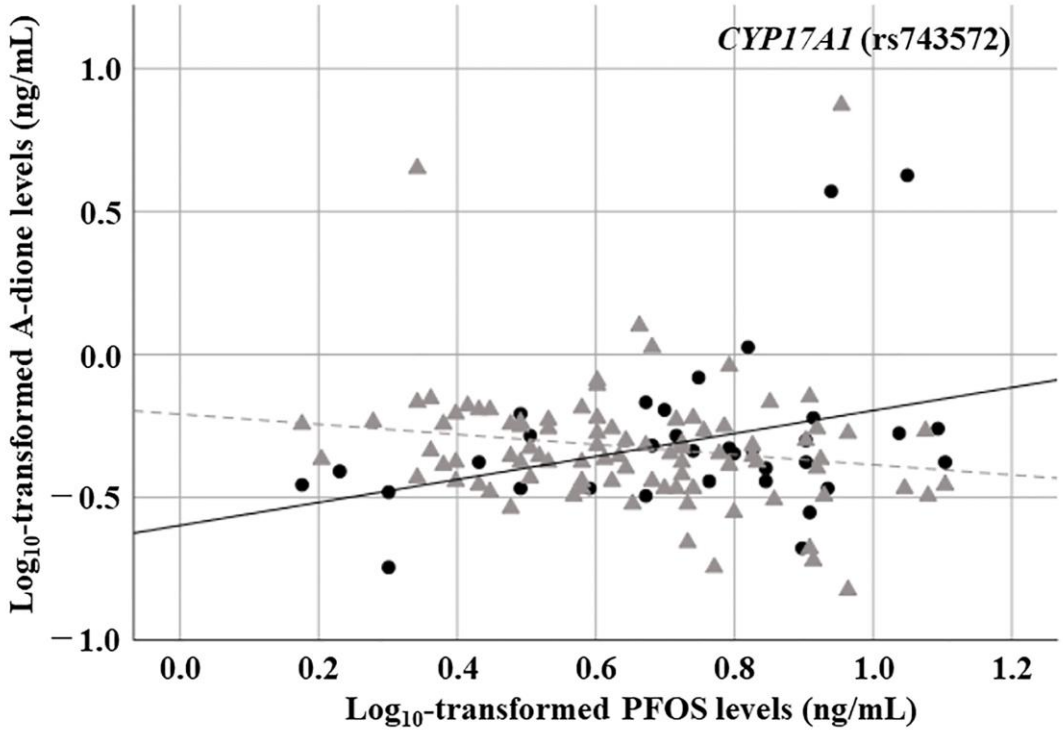
2

1 Fig. 3


rs6203
rs1819698
rs2854964
rs4659175
rs4743709
rs743572
rs10046
rs700519
rs727479
rs605059
rs676387
rs2676531
Block 1 (0 kb)
Block 2 (0 kb)
Block 3 (0 kb)
1 2 3 4 5 6 7 8 9 10 11 12
34
97 2 8 3 91 14
4 10 16 20 11 90 11 12 99
4 1 21 15 13 10 1 11 14
21 2 12 4 13 0 1 11
12 1 3 1 14 1 1
0 2 3 2 15 0
6 0 4 2 14
15 11 3 2
9 11 4
8 10
11

2

1 Fig. 4 (A)



● (Solid line): AA genotype

Log_{10}-transformed A-dione levels (ng/mL)

$= -0.598 + 0.402 \times \text{Log}_{10}$-transformed PFOS levels (ng/mL)

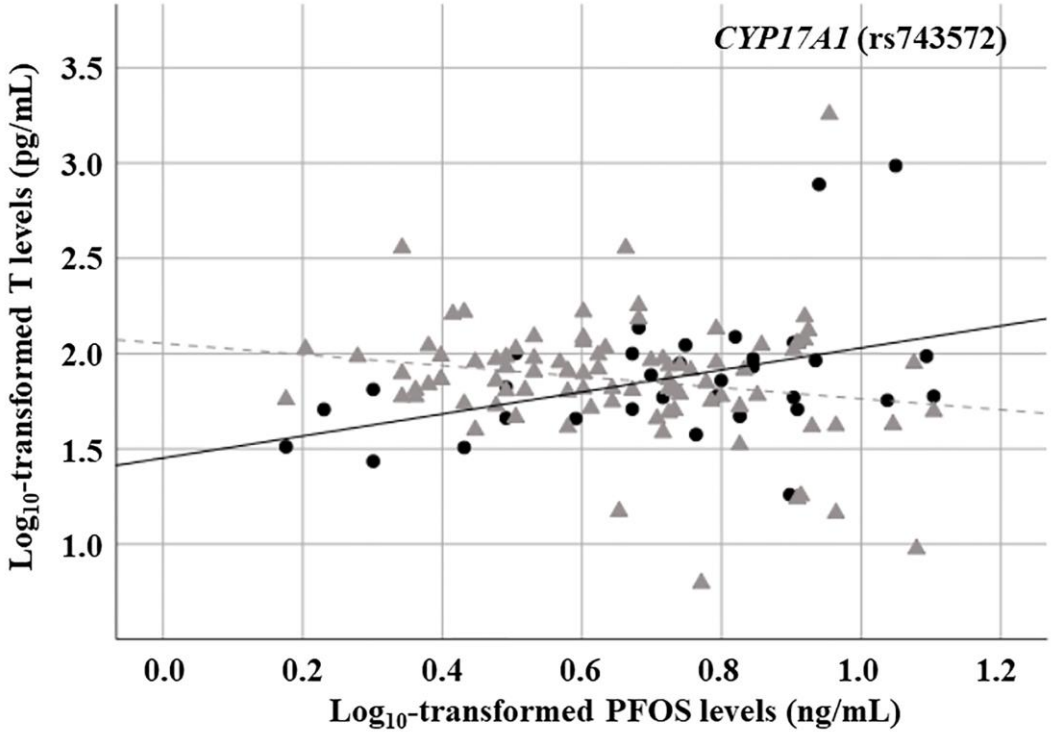
▲ (Dotted line): AG/GG genotype

Log_{10}-transformed A-dione levels (ng/mL)

$= -0.209 - 0.177 \times \text{Log}_{10}$-transformed PFOS levels (ng/mL)

2

Fig. 4 (B)



● (Solid line): AA genotype

Log_{10}-transformed T levels (pg/mL)

$= 1.452 + 0.578 \times Log_{10}$-transformed PFOS levels (ng/mL)

▲ (Dotted line): AG/GG genotype

Log_{10}-transformed T levels (pg/mL)

$= 2.053 - 0.290 \times Log_{10}$-transformed PFOS levels (ng/mL)

Table 1. Characteristics of study participants

Characteristics	Total (n = 224)	Infants		p value[a]
		Males (n = 100)	Females (n = 124)	
Mothers				
Age (years)[b]	30.0 ± 4.8	30.5 ± 4.7	29.5 ± 4.7	0.103
Pre-pregnancy body mass index (BMI) (kg/m^2)[b]	20.4 ± 2.9	20.9 ± 2.6	21.0 ± 3.1	0.809
Smokers during the third trimester of pregnancy[c]				
No	186 (83.0)	87 (87.0)	99 (79.8)	0.156
Yes	38 (17.0)	13 (13.0)	25 (20.2)	
Alcohol consumption during pregnancy[c]				
No	150 (67.0)	69 (69.0)	81 (65.3)	0.561
Yes	74 (33.0)	31 (31.0)	43 (34.7)	
Parity[c]				
Primiparous	116 (51.8)	49 (49.09	67 (54.0)	0.454
Multiparous	108 (48.2)	51 (51.0)	57 (46.0)	
Annual household income (million Japanese yen)[c]				
< 5	154 (68.8)	71 (71.0)	83 (66.9)	0.784
≥ 5	68 (30.4)	28 (28.0)	40 (32.3)	
Missing data	2 (0.9)	1 (1.0)	1 (0.8)	
Maternal blood sampling period[c]				
During pregnancy	153 (68.3)	65 (65.0)	88 (71.0)	0.340
After birth	71 (31.7)	35 (35.0)	36 (29.0)	
Infant sex[c]				
Male	100 (44.6)	100 (100.0)	0 (0.0)	(-)
Female	124 (55.4)	0 (0.0)	124 (100.0)	
Gestational age (weeks)[b]	39.3 ± 1.0	39.2 ± 1.0	39.4 ± 1.1	0.310
Infant birth weight (g)[b]	3,121.9 ± 332.7	3,173.4 ± 306.2	3,080.4 ± 348.4	0.037
Maternal serum levels				
PFOS (ng/mL)[d]	5.0 (3.3, 6.9)	5.1 (3.7, 7.0)	4.8 (3.2, 6.8)	0.417
PFOA (ng/mL)[d]	1.4 (0.9, 2.0)	1.5 (0.9, 2.1)	1.3 (0.8, 1.8)	0.095
Cord serum levels				
P_4 (ng/mL)[d]	217.8 (173.9, 282.4)	228.8 (179.1, 298.7)	206.6 (164.5, 277.7)	0.184
DHEA (ng/mL)[d]	2.3 (1.8, 3.0)	2.1 (1.6, 2.8)	2.4 (2.0, 3.3)	0.002
A-dione (ng/mL)[d]	0.45 (0.36, 0.57)	0.46 (0.36, 0.57)	0.45 (0.36, 0.57)	0.916
DHEA/A-dione[d]	4.8 (3.7, 6.0)	4.2 (3.4, 5.6)	5.0 (4.0, 6.3)	0.003
T (pg/mL)[d]	84.4 (59.5, 111.2)	92.9 (71.0, 117.0)	73.1 (52.9, 99.5)	<0.001
E_2 (ng/mL)[d]	4.8 (3.3, 7.1)	4.7 (3.3, 7.6)	4.8 (3.4, 6.6)	0.458
T/E_2[d]	17.0 (12.1, 22.5)	17.9 (12.9, 25.5)	16.1 (11.9, 21.5)	0.058

[a] Males vs. Females analyzed using the χ^2-test, independent t-test, and Mann-Whitney U-test.

[b] Mean ± standard deviation (SD).

[c] n (%).

[d] Median (inter-quartile range).

Table 2. Fetal genotype frequencies

Gene name, genotype	*n* (%)	HWE	Gene name, genotype	*n* (%)	HWE
CYP17A1 (A>G, dbSNP ID: rs743572)			*HSD3B2* (A>T, dbSNP ID: rs2854964)		
AA	65 (29.0)	$\chi^2 = 2.122$	AA	142 (63.4)	$\chi^2 = 2.173$
AG	101 (45.1)	$p = 0.145$	AT	68 (30.4)	$p = 0.140$
GG	58 (25.9)		TT	14 (6.3)	
CYP19A1 (G>A, dbSNP ID: rs10046)			*HSD3B2* (C>T, dbSNP ID: rs4659175)		
GG	67 (29.9)	$\chi^2 = 1.456$	CC	141 (62.9)	$\chi^2 = 0.210$
GA	119 (53.1)	$p = 0.228$	CT	72 (32.1)	$p = 0.646$
AA	38 (17.0)		TT	11 (4.9)	
CYP19A1 (C>T, dbSNP ID: rs700519)			*HSD17B1* (G>A, dbSNP ID: rs605059)		
CC	138 (61.6)	$\chi^2 = 1.197$	GG	64 (28.6)	$\chi^2 = 0.651$
CT	72 (32.1)	$p = 0.274$	GA	117 (52.2)	$p = 0.420$
TT	14 (6.3)		AA	43 (19.2)	
CYP19A1 (A>C, dbSNP ID: rs727479)			*HSD17B1* (C>A, dbSNP ID: rs676387)		
AA	99 (44.2)	$\chi^2 = 0.885$	CC	67 (29.9)	$\chi^2 = 0.557$
AC	95 (42.4)	$p = 0.347$	CA	116 (51.8)	$p = 0.456$
CC	30 (13.4)		AA	41 (18.3)	
HSD3B1 (T>C, dbSNP ID: rs6203)			*HSD17B1* (C>A, dbSNP ID: rs2676531)		
TT	104 (46.4)	$\chi^2 = 0.069$	CC	63 (28.1)	$\chi^2 = 1.159$
TC	96 (42.9)	$p = 0.793$	CT	119 (53.1)	$p = 0.282$
CC	24 (10.7)		TT	42 (18.8)	
HSD3B2 (C>T, dbSNP ID: rs1819698)			*HSD17B3* (T>C, dbSNP ID: rs4743709)		
CC	85 (37.9)	$\chi^2 = 0.720$	TT	117 (52.2)	$\chi^2 = 2.445$
CT	101 (45.1)	$p = 0.396$	TC	96 (42.9)	$p = 0.118$
TT	38 (17.0)		CC	11 (4.9)	

Abbreviations: *CYP17A1*, cytochrome P450 17A1; *CYP19A1*, cytochrome P450 19A1; *HSD3B1*, 3β-hydroxysteroid dehydrogenase type 1; *HSD3B2*, 3β-hydroxysteroid dehydrogenase type 2; *HSD17B1*, 17β-hydroxysteroid dehydrogenase type 1, and *HSD17B3*, 17β-hydroxysteroid dehydrogenase type 3; HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism.

Chi-squared test was employed to test whether the frequency of genotype distribution conformed to the Hardy-Weinberg equilibrium.

Table 3. Associations between perfluorooctanesulfonic acid (PFOS) or perfluorooctanoic acid (PFOA) levels in maternal sera during pregnancy and infant sex hormone levels in cord sera

	Total (n = 224)				Males (n = 100)				Females (n = 124)			
	Crude[a]		Adjusted[b]		Crude[a]		Adjusted[b]		Crude[a]		Adjusted[b]	
Outcome	β (95% CI)	*p* value	β (95% CI)	*p* value	β (95% CI)	*p* value	β (95% CI)	*p* value	β (95% CI)	*p* value	β (95% CI)	*p* value
Exposure: PFOS (ng/mL)												
P_4 (ng/mL)	-0.311 (-0.499, -0.124)	0.001	-0.400 (-0.598, -0.201)	<0.001	-0.223 (-0.459, 0.013)	0.063	-0.270 (-0.512, -0.029)	0.029	-0.401 (-0.684, -0.118)	0.006	-0.517 (-0.829, -0.205)	0.001
DHEA (ng/mL)	0.315 (0.134, 0.496)	0.001	0.359 (0.167, 0.552)	<0.001	0.252 (0.027, 0.477)	0.029	0.249 (0.015, 0.483)	0.037	0.393 (0.124, 0.663)	0.005	0.448 (0.146, 0.751)	0.004
A-dione (ng/mL)	-0.013 (-0.148, 0.122)	0.852	0.022 (-0.123, 0.167)	0.767	-0.056 (-0.243, 0.131)	0.555	-0.049 (-0.239, 0.142)	0.614	0.025 (-0.170, 0.221)	0.798	0.077 (-0.141, 0.296)	0.485
DHEA/A-dione	0.328 (0.116, 0.540)	0.003	0.338 (0.110, 0.565)	0.004	0.308 (0.046, 0.570)	0.022	0.298 (0.026, 0.569)	0.032	0.368 (0.048, 0.688)	0.025	0.371 (0.012, 0.730)	0.043
T (pg/mL)	-0.049 (-0.227, 0.128)	0.585	0.007 (-0.180, 0.194)	0.945	-0.142 (-0.373, 0.088)	0.223	-0.091 (-0.334, 0.151)	0.457	0.007 (-0.253, 0.267)	0.958	0.096 (-0.194, 0.385)	0.514
E_2 (ng/mL)	0.217 (0.065, 0.369)	0.005	0.166 (0.005, 0.326)	0.044	0.272 (0.035, 0.508)	0.025	0.213 (-0.031, 0.458)	0.087	0.164 (-0.037, 0.365)	0.108	0.121 (-0.102, 0.343)	0.284
T/E_2	-0.266 (-0.417, -0.115)	0.001	-0.159 (-0.313, -0.005)	0.043	-0.414 (-0.614, -0.214)	<0.001	-0.305 (-0.497, -0.112)	0.002	-0.157 (-0.377, 0.062)	0.159	-0.025 (-0.263, 0.212)	0.833
Exposure: PFOA (ng/mL)												
P_4 (ng/mL)	0.174 (0.025, 0.323)	0.023	0.160 (-0.018, 0.338)	0.077	0.174 (-0.024, 0.373)	0.085	0.216 (-0.026, 0.457)	0.080	0.156 (-0.062, 0.374)	0.159	0.111 (-0.151, 0.374)	0.402
DHEA (ng/mL)	-0.121 (-0.267, 0.024)	0.101	-0.161 (-0.332, 0.010)	0.065	-0.011 (-0.205, 0.183)	0.911	-0.036 (-0.274, 0.201)	0.763	-0.162 (-0.370, 0.046)	0.126	-0.202 (-0.452, 0.049)	0.114
A-dione (ng/mL)	-0.056 (-0.161, 0.050)	0.302	-0.064 (-0.190, 0.062)	0.318	-0.018 (-0.175, 0.140)	0.823	0.019 (-0.171, 0.208)	0.844	-0.079 (-0.225, 0.068)	0.289	-0.096 (-0.272, 0.080)	0.280
DHEA/A-dione	-0.066 (-0.235, 0.104)	0.446	-0.097 (-0.298, 0.104)	0.343	0.007 (-0.219, 0.233)	0.953	-0.055 (-0.331, 0.221)	0.694	-0.083 (-0.329, 0.163)	0.505	-0.105 (-0.400, 0.189)	0.481
T (pg/mL)	-0.004 (-0.143, 0.136)	0.959	0.013 (-0.150, 0.176)	0.877	-0.075 (-0.270, 0.119)	0.443	0.028 (-0.213, 0.269)	0.817	0.013 (-0.183, 0.209)	0.899	0.014 (-0.220, 0.248)	0.904
E_2 (ng/mL)	0.097 (-0.024, 0.218)	0.117	0.004 (-0.137, 0.145)	0.954	0.133 (-0.069, 0.335)	0.194	0.034 (-0.212, 0.281)	0.782	0.062 (-0.090, 0.215)	0.422	-0.014 (-0.195, 0.166)	0.877
T/E_2	-0.100 (-0.222, 0.021)	0.105	0.009 (-0.127, 0.144)	0.900	-0.209 (-0.386, -0.032)	0.021	-0.006 (-0.207, 0.195)	0.950	-0.050 (-0.216, 0.117)	0.557	0.028 (-0.163, 0.220)	0.770

Associations between maternal PFOS or PFOA levels and infant sex hormone levels were evaluated using multiple linear regression models.

[a] Crude: Non-adjusted.

[b] Adjusted: Adjusted for maternal age (years; continuous), maternal smoking during the third trimester (yes/no), maternal alcohol consumption during pregnancy (yes/no), annual household income (< 5/≥ 5 million Japanese yen), parity (primipara/multipara), infant sex (male/female; only all participants), maternal blood sampling periods (during pregnancy or after birth), and infant birth weight (grams; continuous).

β (95% CI) represents change (95% confidence intervals) in $\log_{10}$-transformed P_4 (ng/mL), DHEA (ng/mL), A-dione (ng/mL), DHEA/A-dione, T (pg/mL), E_2 (ng/mL), or T/E_2 levels for each 10-fold PFOS or PFOA level (ng/mL).

Table 4. Associations between PFOS levels in maternal sera during pregnancy and infant genotypes *CYP17A1* (rs743572) on sex hormone levels in cord sera in female infants

		Females (n = 124)			
		Crude[a]		Adjusted[b]	
Outcome	Exposure/Genotype	β (95% CI)	p value	β (95% CI)	p value
P_4	PFOS (ng/mL)	-0.316 (-0.811, 0.178)	0.207	-0.392 (-0.894, 0.111)	0.126
(ng/mL)	*CYP17A1* (rs743572)-AG/GG (vs. AA)	0.045 (-0.405, 0.495)	0.842	0.095 (-0.361, 0.552)	0.680
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	-0.162 (-0.770, 0.445)	0.598	-0.218 (-0.831, 0.394)	0.482
DHEA	PFOS (ng/mL)	0.481 (0.008, 0.953)	0.046	0.512 (0.023, 1.002)	0.040
(ng/mL)	*CYP17A1* (rs743572)-AG/GG (vs. AA)	0.121 (-0.309, 0.551)	0.579	0.103 (-0.342, 0.547)	0.648
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	-0.110 (-0.691, 0.470)	0.707	-0.084 (-0.680, 0.513)	0.782
A-dione	PFOS (ng/mL)	0.402 (0.071, 0.734)	0.018	0.445 (0.102, 0.787)	0.012
(ng/mL)	*CYP17A1* (rs743572)-AG/GG (vs. AA)	0.390 (0.087, 0.692)	0.012	0.396 (0.084, 0.707)	0.013
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	-0.579 (-0.987, -0.171)	0.006	-0.579 (-0.997, -0.161)	0.007
DHEA/A-dione	PFOS (ng/mL)	0.078 (-0.478, 0.634)	0.781	0.068 (-0.508, 0.643)	0.816
	CYP17A1 (rs743572)-AG/GG (vs. AA)	-0.269 (-0.775, 0.238)	0.295	-0.293 (-0.815, 0.229)	0.269
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	0.469 (-0.215, 1.153)	0.177	0.495 (-0.206, 1.196)	0.164
T	PFOS (ng/mL)	0.578 (0.139, 1.016)	0.010	0.641 (0.191, 1.091)	0.006
(pg/mL)	*CYP17A1* (rs743572)-AG/GG (vs. AA)	0.601 (0.202, 1.000)	0.003	0.595 (0.186, 1.003)	0.005
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	-0.868 (-1.407, -0.329)	0.002	-0.856 (-1.404, -0.307)	0.003
E_2	PFOS (ng/mL)	0.388 (0.040, 0.736)	0.029	0.359 (0.004, 0.714)	0.048
(ng/mL)	*CYP17A1* (rs743572)-AG/GG (vs. AA)	0.213 (-0.103, 0.530)	0.185	0.243 (-0.080, 0.565)	0.139
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	-0.358 (-0.786, 0.070)	0.100	-0.383 (-0.815, 0.050)	0.083
T/E_2	PFOS (ng/mL)	0.190 (-0.188, 0.567)	0.322	0.282 (-0.096, 0.659)	0.142
	CYP17A1 (rs743572)-AG/GG (vs. AA)	0.388 (0.044, 0.731)	0.027	0.352 (0.009, 0.695)	0.044
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	-0.510 (-0.975, -0.046)	0.032	-0.473 (-0.933, -0.013)	0.044

Associations between maternal PFOS levels and infant genotypes on sex hormone levels were evaluated using multiple linear regression models.

[a] Crude: Non-adjusted.

[b] Adjusted: Adjusted for maternal age (years; continuous), maternal smoking during the third trimester (yes/no), maternal alcohol consumption during pregnancy (yes/no), annual household income (< 5/≥ 5 million Japanese yen), parity (primipara/multipara), infant sex (male/female; all participants), maternal blood sampling periods (during pregnancy or after birth), and infant birth weight (grams; continuous).

β (95% CI) represents change (95% confidence intervals) in $\log_{10}$-transformed A-dione (ng/mL), T (pg/mL), E_2 (ng/mL), or DHEA/A-dione levels for each 10-fold PFOS level (ng/mL).

PFOS-*CYP17A1* (rs743572) interaction term was defined as "$\log_{10}$-transformed PFOS levels (continuous) * genotype (0 = AA and 1 =AG/GG)".

Table 5. PFOS levels in maternal sera and sex steroid hormone levels in cord sera stratified by female infant genotypes *CYP17A1* (rs743572)

		Exposure: PFOS (ng/mL)			
		Females (n = 124)			
Outcome	*CYP17A1* (rs743572) Infant genotype	Crude[a] β (95% CI)	*p* value	Adjusted[b] β (95% CI)	*p* value
P_4 (ng/mL)	AA	-0.316 (-0.892, 0.259)	0.271	-0.513 (-1.124, 0.097)	0.096
	AG/GG	-0.479 (-0.816, -0.142)	0.006	-0.558 (-0.929, -0.186)	0.004
DHEA (ng/mL)	AA	0.481 (-0.018, 0.979)	0.058	0.588 (0.024, 1.152)	0.042
	AG/GG	0.370 (0.034, 0.706)	0.031	0.379 (-0.002, 0.761)	0.051
A-dione (ng/mL)	AA	0.402 (0.025, 0.780)	0.038	0.494 (0.059, 0.930)	0.028
	AG/GG	-0.177 (-0.405, 0.052)	0.128	-0.157 (-0.419, 0.104)	0.235
DHEA/A-dione	AA	0.078 (-0.413, 0.570)	0.748	0.093 (-0.461, 0.647)	0.731
	AG/GG	0.547 (0.128, 0.965)	0.011	0.536 (0.065, 1.008)	0.026
T (pg/mL)	AA	0.578 (0126, 1.029)	0.014	0.679 (0.176, 1.181)	0.010
	AG/GG	-0.290 (-0.605, 0.025)	0.071	-0.198 (-0.554, 0.157)	0.271
E_2 (ng/mL)	AA	0.388 (-0.014, 0.790)	0.058	0.360 (-0.117, 0.838)	0.132
	AG/GG	0.030 (-0.208, 0.268)	0.801	-0.017 (-0.285, 0.251)	0.900
T/E_2	AA	0.190 (-0.200, 0.580)	0.330	0.318 (-0.099, 0.735)	0.128
	AG/GG	-0.320 (-0.591, -0.049)	0.021	-0.181 (-0.485, 0.122)	0.239

Associations between maternal PFOS and sex hormone levels were evaluated using multiple linear regression models.

[a] Crude: Non-adjusted.

[b] Adjusted: Adjusted for maternal age (years; continuous), maternal smoking during the third trimester (yes/no), maternal alcohol consumption during pregnancy (yes/no), annual household income (< 5/≥ 5 million Japanese yen), parity (primipara/multipara), infant sex (male/female; all participants), maternal blood sampling periods (during pregnancy or after birth), and infant birth weight (grams; continuous).

β (95% CI) represents change (95% confidence intervals) in $\log_{10}$-transformed P_4 (ng/mL), DHEA (ng/mL), A-dione (ng/mL), DHEA/A-dione, T (pg/mL), E_2 (ng/mL), or T/E2 levels per 10-fold increase in maternal PFOS levels.

Supplementary Material

Associations among maternal perfluoroalkyl substance levels, fetal sex-hormone enzymatic gene polymorphisms, and fetal sex hormone levels in the Hokkaido study

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Supplementary Methods:

1. Pre-amplification for high-throughput PCR sequencing

Pre-amplification was performed on each sample using Qiagen 2× Multiplex PCR Master Mix according to the manufacturer's protocol (Qiagen GmbH, Hilden, Germany; Fluidigm Corp., South San Francisco, CA, USA) to increase the amount of available template, including 96 assays used in this system. The mixture (final volume 5.0 μL) consisted of 2.5 μL Qiagen 2× Multiplex PCR master mix, 0.5 μL of 10× SNP type-specific target amplification (STA) primer pool, comprising 96 μL/100 μM each of SNP-type assay STA primer and LSP, and 208.0 μL of DNA suspension buffer (TEKnova, Hollister, CA, USA), 1.25 μL genome DNA, and water. Pre-amplifications were conducted in a Verity 96-well Thermal Cycler (Applied Biosystems, Foster City, CA, USA) according to the following protocol: initial denaturation at 95℃ for 15 min; 14 cycles of 15 s at 95℃ and 4 min at 60℃. Each pre-amplification product was diluted 100× before use as a template in the subsequent PCR reactions.

2. High-throughput real-time PCR using dynamic chips

Fluidigm 96.96 real-time PCR was performed according to the manufacturer's instructions (Fluidigm Corp., South San Francisco, CA, USA). Sample and assay mixtures were prepared separately. Each sample mixture (final volume: 6.0 μL) consisted of 3.0 μL of the Biotium 2× fast probe master mix (Biotium Inc., Fremont, CA, USA), 0.3 μL of the 20× SNP type sample loading reagent (Fluidigm Corp.), 0.1 μL of the 60× SNP type reagent (Fluidigm Corp.), 0.036 μL of the 50× ROX solution (Invitrogen, Waltham, MA, US), 0.064 μL of water, and 2.5 μL of the diluted pre-amplification product as the template. The assay mixture for each sample (final volume 5.0 μL) consisted of 2.5 μL of the 2× assay loading reagent (Fluidigm Corp.), 1.5 μL of water, and 1.0 μL of the SNP type assay mix, comprising 3.0 μL of the SNP type assay ASP1/ASP2 (Fluidigm Corp.), 8.0 μL of the SNP type assay LSP (Fluidigm Corp.), and 29.0 μL of the DNA suspension buffer (TEKnova). The assay (5 μL) and the sample (6 μL) were added to each assay and sample

inlet, respectively, and were loaded into separate reaction chambers on a 96.96 Dynamic Array IFC chip (Fluidigm Corp.) on an IFC controller HX (Fluidigm Corp.). The protocol was as follows: 95℃ initial denaturation for 5 min; 1 cycle of 15 s at 95℃, 45 s at 64℃, and 15 s at 72℃; 1 cycle of 15 s at 95℃, 45 s at 63℃, and 15 s at 72℃; 1 cycle of 15 s at 95℃, 45 s at 62℃, and 15 s at 72℃; 1 cycle of 15 s at 95℃, 45 s at 61℃, and 15 s at 72℃; and 34 cycles of 15 s at 95℃, 45 s at 60℃, and 15 s at 72℃ using the FC1 cycler (Fluidigm Corp.). Fluorescence was measured using an EP1 reader (Fluidigm Corp.) coupled to the SNP genotyping analytical software v.3.0.2. (Fluidigm Corp.). Only the infant genotypes that were successfully tested in duplicate were used.

3. TaqMan real-time PCR

Five genotyping experiments were performed using the StepOne real-time PCR system (Applied Biosystems) and a fluorogenic 5'-nuclease assay with TaqMan minor groove binder probes (Applied Biosystems) according to the manufacturer's protocol. Each reaction mixture (final volume: 10 μL) consisted of 2 ng/μL of the genomic DNA, TaqMan Assay on-Demand SNP genotyping assay mix (Applied Biosystems), TaqMan GTXpress Master Mix (Applied Biosystems), and No AmpErase UNG (Applied Biosystems). The reaction conditions were 20 s at 95.0℃, 40 cycles of 3 s at 95℃, and 20 s at 60℃. Allelic discrimination was determined by measuring the relative dye fluorescence at 60℃. Only infant genotypes that were successfully tested in duplicates were used.

Supplementary Table 1. PFAS levels during pregnancy of included participants (n = 224) and excluded participants (n = 223)

	Included participants (n = 224)	Excluded participants (n = 223)	p value
Maternal serum levels			
PFOS (ng/mL)	5.0 (3.3, 6.9)	5.5 (3.6, 7.2)	0.174
PFOA (ng/mL)	1.4 (0.9, 2.0)	1.2 (0.8, 1.6)	0.003

Median (inter-quartile range).

Mann-Whitney's U-test.

Supplementary Table 2. Spearman's rank correlation coefficients between maternal gestational weeks for blood sample collection and PFOS, PFOA, and sex hormone levels

	Gestational weeks at blood sample collection (weeks)	
	Spearman's ρ	*p* value
PFOS (ng/mL)	0.046	0.497
PFOA (ng/mL)	0.075	0.266
P_4 (ng/mL)	0.011	0.871
DHEA (ng/mL)	0.056	0.408
A-dione (ng/mL)	0.068	0.309
DHEA/A-dione	-0.042	0.536
T (pg/mL)	-0.058	0.386
E_2 (ng/mL)	0.041	0.543
T/E_2	-0.081	0.229

Supplementary Table 3. Sensitivity analysis of associations between perfluorooctanesulfonic acid (PFOS) or perfluorooctanoic acid (PFOA) levels in maternal sera during pregnancy and infant sex hormone levels in cord sera among pregnant women and infant pairs for which maternal blood samples were obtained before delivery

	Total (n = 153)				Males (n = 65)				Females (n = 88)			
	Crude[a]		Adjusted[b]		Crude[a]		Adjusted[b]		Crude[a]		Adjusted[b]	
Outcome	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Exposure: PFOS (ng/mL)												
P_4 (ng/mL)	-0.376 (-0.612, -0.141)	0.002	-0.463 (-0.709, -0.217)	<0.001	-0.263 (-0.572, 0.046)	0.094	-0.301 (-0.644, 0.042)	0.084	-0.479 (-0.809, -0.148)	0.005	-0.542 (-0.897, -0.187)	0.003
DHEA (ng/mL)	0.361 (0.135, 0.587)	0.002	0.440 (0.208, 0.672)	<0.001	0.386 (0.104, 0.668)	0.008	0.450 (0.163, 0.736)	0.003	0.394 (0.078, 0.711)	0.015	0.433 (0.087, 0.780)	0.015
A-dione (ng/mL)	-0.074 (-0.237, 0.090)	0.373	-0.024 (-0.198, 0.149)	0.781	-0.038 (-0.283, 0.207)	0.757	-0.016 (-0.275, 0.244)	0.905	-0.095 (-0.319, 0.129)	0.400	-0.048 (-0.290, 0.195)	0.697
DHEA/A-dione	0.435 (0.160, 0.709)	0.002	0.465 (0.177, 0.753)	0.002	0.424 (0.078, 0.770)	0.017	0.465 (0.083, 0.848)	0.018	0.490 (0.101, 0.878)	0.014	0.481 (0.057, 0.906)	0.027
T (pg/mL)	-0.044 (-0.262, 0.174)	0.692	-0.015 (-0.245, 0.215)	0.896	-0.011 (-0.328, 0.305)	0.944	0.042 (-0.312, 0.396)	0.813	-0.092 (-0.389, 0.206)	0.542	-0.035 (-0.358, 0.288)	0.829
E_2 (ng/mL)	0.207 (0.032, 0.381)	0.020	0.192 (0.008, 0.375)	0.040	0.412 (0.126, 0.699)	0.005	0.386 (0.076, 0.695)	0.016	0.077 (-0.144, 0.298)	0.490	0.074 (-0.162, 0.310)	0.536
T/E_2	-0.251 (-0.441, -0.060)	0.010	-0.207 (-0.400, -0.013)	0.036	-0.423 (-0.668, -0.178)	0.001	-0.344 (-0.594, -0.093)	0.008	-0.169 (-0.442, 0.104)	0.222	-0.109 (-0.395, 0.178)	0.452
Exposure: PFOA (ng/mL)												
P_4 (ng/mL)	0.104 (-0.079, 0.286)	0.263	0.053 (-0.158, 0.264)	0.621	0.167 (-0.091, 0.425)	0.200	0.235 (-0.079, 0.549)	0.140	0.022 (-0.232, 0.374)	0.861	-0.033 (-0.330, 0.265)	0.827
DHEA (ng/mL)	-0.128 (-0.303, 0.046)	0.149	-0.124 (-0.322, 0.075)	0.220	-0.106 (-0.351, 0.139)	0.391	-0.089 (-0.370, 0.192)	0.529	-0.068 (-0.308, 0.173)	0.578	-0.111 (-0.395, 0.174)	0.441
A-dione (ng/mL)	-0.060 (-0.183, 0.063)	0.336	-0.052 (-0.194, 0.090)	0.468	-0.023 (-0.226, 0.179)	0.819	0.082 (-0.153, 0.317)	0.486	-0.080 (-0.245, 0.084)	0.334	-0.086 (-0.278, 0.106)	0.377
DHEA/A-dione	-0.068 (-0.281, 0.145)	0.527	-0.071 (-0.316, 0.173)	0.564	-0.083 (-0.381, 0.216)	0.582	-0.171 (-0.534, 0.191)	0.348	0.013 (-0.283, 0.309)	0.931	-0.025 (-0.372, 0.322)	0.886
T (pg/mL)	0.020 (-0.145, 0.185)	0.810	0.013 (-0.176, 0.202)	0.889	-0.038 (-0.300, 0.223)	0.770	0.035 (-0.287, 0.356)	0.829	-0.002 (-0.221, 0.218)	0.988	0.010 (-0.247, 0.256)	0.940
E_2 (ng/mL)	0.043 (-0.091, 0.176)	0.530	-0.012 (-0.165, 0.141)	0.877	0.122 (-0.128, 0.372)	0.334	0.090 (-0.206, 0.385)	0.545	-0.014 (-0.177, 0.150)	0.869	-0.045 (-0.232, 0.143)	0.635
T/E_2	-0.023 (-0.169, 0.124)	0.762	0.025 (-0.136, 0.186)	0.756	-0.160 (-0.378, 0.057)	0.145	-0.055 (-0.297, 0.187)	0.651	0.012 (-0.191, 0.214)	0.908	0.055 (-0.173, 0.282)	0.635

Associations between maternal PFOS or PFOA levels and infant sex hormone levels were evaluated using multiple linear regression models.

[a] Crude: Non-adjusted.

[b] Adjusted: Adjusted for maternal age (years; continuous), maternal smoking during the third trimester (yes/no), maternal alcohol consumption during pregnancy (yes/no), annual household income (< 5/≥ 5 million Japanese yen), parity (primipara/multipara), infant sex (male/female; only all participants), maternal blood sampling periods (during pregnancy or after birth), and infant birth weight (grams; continuous).

β (95% CI) represents change (95% confidence intervals) in $\log_{10}$-transformed P_4 (ng/mL), DHEA (ng/mL), A-dione (ng/mL), DHEA/A-dione, T (pg/mL), E_2 (ng/mL), or T/E_2 levels for each 10-fold PFOS or PFOA level (ng/mL).

Supplementary Table 4. Associations between PFOS or PFOA levels in maternal sera during pregnancy and infant sex on sex hormone levels in cord sera

		Total (n = 224)			
		Crude[a]		Adjusted[b]	
Outcome	Exposure/Infant sex	β (95% CI)	p value	β (95% CI)	p value
P_4	PFOS	-0.223 (-0.501, 0.054)	0.115	-0.301 (-0.583, -0.019)	0.037
(ng/mL)	Females (vs. Males)	0.049 (-0.222, 0.319)	0.723	0.044 (-0.226, 0.190)	0.747
	PFOS × Sex (Interaction term)	-0.178 (-0.554, 0.198)	0.353	-0.186 (-0.562, 0.190)	0.331
DHEA	PFOS	0.252 (-0.013, 0.517)	0.062	0.269 (-0.004, 0.543)	0.054
(ng/mL)	Females (vs. Males)	0.022 (-0.236, 0.280)	0.869	0.004 (-0.258, 0.266)	0.975
	PFOS × Sex (Interaction term)	0.141 (-0.217, 0.500)	0.438	0.169 (-0.196, 0.534)	0.361
A-dione	PFOS	0.056 (-0.257, 0.145)	0.585	-0.041 (-0.247, 0.166)	0.698
(ng/mL)	Females (vs. Males)	-0.046 (-0.241, 0.150)	0.647	-0.063 (-0.261, 0.135)	0.531
	PFOS × Sex (Interaction term)	0.081 (-0.191, 0.353)	0.558	0.117 (-0.158, 0.392)	0.402
DHEA/A-dione	PFOS	0.308 (-0.005, 0.620)	0.053	0.310 (-0.014, 0.633)	0.061
	Females (vs. Males)	0.067 (-0.237, 0.372)	0.664	0.067 (-0.243, 0.377)	0.671
	PFOS × Sex (Interaction term)	0.060 (-0.363, 0.483)	0.779	0.052 (-0.380, 0.484)	0.812
T	PFOS	-0.142 (-0.403, 0.118)	0.283	-0.096 (-0.362, 0.169)	0.476
(pg/mL)	Females (vs. Males)	-0.202 (-0.455, 0.052)	0.119	-0.218 (-0.473, 0.037)	0.093
	PFOS × Sex (Interaction term)	0.149 (-0.204, 0.502)	0.406	0.193 (-0.161, 0.548)	0.284
E_2	PFOS	0.272 (0.046, 0.498)	0.019	0.220 (-0.008, 0.449)	0.059
(ng/mL)	Females (vs. Males)	0.042 (-0.178, 0.262)	0.706	0.031 (-0.189, 0.250)	0.784
	PFOS × Sex (Interaction term)	-0.108 (-0.414, 0.199)	0.489	-0.103 (-0.408, 0.202)	0.506
T/E_2	PFOS	-0.412 (-0.636, -0.192)	<0.001	-0.317 (-0.534, -0.100)	0.004
	Females (vs. Males)	-0.244 (-0.460, -0.027)	0.027	-0.249 (-0.457, -0.041)	0.019
	PFOS × Sex (Interaction term)	0.257 (-0.044, 0.558)	0.094	0.296 (0.007, 0.586)	0.045
P_4	PFOA	0.174 (-0.063, 0.412)	0.149	0.169 (-0.095, 0.432)	0.208
(ng/mL)	Females (vs. Males)	-0.053 (-0.145, 0.038)	0.251	-0.052 (-0.147, 0.043)	0.280
	PFOA × Sex (Interaction term)	-0.018 (-0.325, 0.288)	0.907	-0.015 (-0.331, 0.301)	0.927
DHEA	PFOA	-0.011 (-0.239, 0.217)	0.924	-0.069 (-0.323, 0.184)	0.591
(ng/mL)	Females (vs. Males)	0.121 (0.033, 0.209)	0.007	0.109 (0.018, 0.201)	0.020
	PFOA × Sex (Interaction term)	-0.151 (-0.445, 0.144)	0.314	-0.149 (-0.454, 0.155)	0.334
A-dione	PFOA	-0.018 (-0.186, 0.151)	0.836	-0.018 (-0.204, 0.169)	0.853
(ng/mL)	Females (vs. Males)	0.014 (-0.052, 0.079)	0.682	0.020 (-0.048, 0.087)	0.569
	PFOA × Sex (Interaction term)	-0.061 (-0.279, 0.157)	0.582	-0.075 (-0.299, 0.148)	0.507
DHEA/A-dione	PFOA	0.007 (-0.261, 0.275)	0.960	-0.052 (-0.350, 0.247)	0.734
	Females (vs. Males)	0.107 (0.004, 0.211)	0.042	0.090 (-0.018, 0.197)	0.102
	PFOA × Sex (Interaction term)	-0.090 (-0.437, 0.257)	0.610	-0.074 (-0.432, 0.284)	0.684
T	PFOA	-0.075 (-0.295, 0.144)	0.498	-0.017 (-0.259, 0.224)	0.887
(pg/mL)	Females (vs. Males)	-0.109 (-0.194, -0.025)	0.012	-0.091 (-0.178, -0.004)	0.041
	PFOA × Sex (Interaction term)	0.088 (-0.195, 0.371)	0.541	0.049 (-0.240, 0.339)	0.738
E_2	PFOA	0.133 (-0.059, 0.326)	0.173	0.046 (-0.163, 0.256)	0.664
(ng/mL)	Females (vs. Males)	-0.023 (-0.097, 0.051)	0.540	-0.038 (-0.114, 0.037)	0.317
	PFOA × Sex (Interaction term)	-0.071 (-0.320, 0.177)	0.573	-0.068 (-0.319, 0.183)	0.592
T/E_2	PFOA	-0.209 (-0.400, -0.018)	0.032	-0.064 (-0.264, 0.137)	0.532
	Females (vs. Males)	-0.086 (-0.160, -0.012)	0.022	-0.053 (-0.125, 0.020)	0.152
	PFOA × Sex (Interaction term)	0.159 (-0.088, 0.406)	0.205	0.118 (-0.122, 0.358)	0.335

Associations between maternal PFOS or PFOA levels and infant sex on sex hormone levels were evaluated using multiple linear regression models.

[a] Crude: Non-adjusted.

[b] Adjusted: Adjusted for maternal age (years; continuous), maternal smoking during the third trimester (yes/no), maternal alcohol consumption during pregnancy (yes/no), annual household

income (< 5/≥ 5 million Japanese yen), parity (primipara/multipara), maternal blood sampling periods (during pregnancy or after birth), and infant birth weight (grams; continuous).

β (95% CI) represents change (95% confidence intervals) in $\log_{10}$-transformed A-dione (ng/mL), T (pg/mL), E_2 (ng/mL), or DHEA/A-dione levels for each 10-fold PFOS or PFOA level (ng/mL).

PFAS-sex interaction term was defined as "$\log_{10}$-transformed PFOS or PFOA levels (continuous) * sex (0 = males and 1 = females)".

Supplementary Table 5. Sensitivity analysis of associations between PFOS or PFOA levels in maternal sera during pregnancy and infant sex on sex hormone levels in cord sera among pregnant women and infant pairs for which maternal blood samples were obtained before delivery

		Total (n = 153)			
		Crude[a]		Adjusted[b]	
Outcome	Exposure/Infant sex	β (95% CI)	p value	β (95% CI)	p value
P_4	PFOS	-0.263 (-0.646, 0.120)	0.177	-0.337 (-0.732, 0.058)	0.094
(ng/mL)	Females (vs. Males)	0.024 (-0.332, 0.381)	0.894	0.013 (-0.349, 0.375)	0.945
	PFOS × Sex (Interaction term)	-0.216 (-0.697, 0.266)	0.378	-0.200 (-0.690, 0.290)	0.421
DHEA	PFOS	0.386 (0.023, 0.748)	0.037	0.410 (0.036, 0.783)	0.032
(ng/mL)	Females (vs. Males)	0.163 (-0.175, 0.500)	0.342	0.131 (-0.211, 0.473)	0.451
	PFOS × Sex (Interaction term)	0.009 (-0.447, 0.464)	0.970	0.048 (-0.415, 0.511)	0.838
A-dione	PFOS	-0.038 (-0.310, 0.234)	0.782	-0.015 (-0.293, 0.264)	0.918
(ng/mL)	Females (vs. Males)	0.038 (-0.216, 0.291)	0.769	0.006 (-0.249, 0.261)	0.963
	PFOS × Sex (Interaction term)	-0.057 (-0.399, 0.285)	0.742	-0.016 (-0.361, 0.330)	0.929
DHEA/A-dione	PFOS	0.424 (-0.021, 0.869)	0.062	0.424 (-0.039, 0.888)	0.072
	Females (vs. Males)	0.125 (-0.289, 0.539)	0.552	0.125 (-0.300, 0.549)	0.562
	PFOS × Sex (Interaction term)	0.066 (-0.493, 0.625)	0.816	0.064 (-0.511, 0.638)	0.827
T	PFOS	-0.011 (-0.370, 0.347)	0.951	0.028 (-0.342, 0.398)	0.882
(pg/mL)	Females (vs. Males)	-0.044 (-0.377, 0.290)	0.796	-0.055 (-0.395, 0.284)	0.748
	PFOS × Sex (Interaction term)	-0.081 (-0.531, 0.370)	0.724	-0.068 (-0.528, 0.391)	0.769
E_2	PFOS	0.412 (0.126, 0.699)	0.005	0.385 (0.093, 0.677)	0.010
(ng/mL)	Females (vs. Males)	0.201 (-0.066, 0.467)	0.139	0.170 (-0.097, 0.438)	0.210
	PFOS × Sex (Interaction term)	-0.335 (-0.695, 0.025)	0.068	-0.307 (-0.669, 0.055)	0.095
T/E_2	PFOS	-0.423 (-0.737, -0.110)	0.008	-0.357 (-0.667, -0.048)	0.024
	Females (vs. Males)	-0.244 (-0.536, 0.047)	0.100	-0.226 (-0.509, 0.058)	0.118
	PFOS × Sex (Interaction term)	0.254 (-0.139, 0.648)	0.204	0.239 (-0.145, 0.623)	0.220
P_4	PFOA	0.167 (-0.162, 0.496)	0.316	0.137 (-0.219, 0.494)	0.447
(ng/mL)	Females (vs. Males)	-0.085 (-0.208, 0.038)	0.174	-0.094 (-0.221, 0.033)	0.145
	PFOA × Sex (Interaction term)	-0.145 (-0.542, 0.252)	0.472	-0.121 (-0.533, 0.290)	0.561
DHEA	PFOA	-0.106 (-0.417, 0.205)	0.502	-0.120 (-0.455, 0.215)	0.479
(ng/mL)	Females (vs. Males)	0.141 (0.024, 0.257)	0.018	0.144 (0.024, 0.263)	0.019
	PFOA × Sex (Interaction term)	0.038 (-0.337, 0.414)	0.841	-0.005 (-0.392, 0.382)	0.979
A-dione	PFOA	-0.023 (-0.248, 0.202)	0.838	0.012 (-0.227, 0.251)	0.921
(ng/mL)	Females (vs. Males)	0.002 (-0.082, 0.086)	0.967	0.005 (-0.080, 0.090)	0.904
	PFOA × Sex (Interaction term)	-0.057 (-0.329, 0.215)	0.678	-0.092 (-0.368, 0.184)	0.510
DHEA/A-dione	PFOA	-0.083 (-0.464, 0.299)	0.670	-0.132 (-0.544, 0.280)	0.527
	Females (vs. Males)	0.139 (-0.004, 0.282)	0.057	0.138 (-0.008, 0.285)	0.064
	PFOA × Sex (Interaction term)	0.095 (-0.366, 0.556)	0.683	0.087 (-0.388, 0.563)	0.717
T	PFOA	-0.038 (-0.335, 0.258)	0.798	0.018 (-0.300, 0.337)	0.909
(pg/mL)	Females (vs. Males)	-0.106 (-0.217, 0.005)	0.061	-0.101 (-0.214, 0.013)	0.081
	PFOA × Sex (Interaction term)	0.037 (-0.322, 0.395)	0.840	-0.007 (-0.375, 0.361)	0.969
E_2	PFOA	0.122 (-0.121, 0.365)	0.323	0.086 (-0.171, 0.342)	0.510
(ng/mL)	Females (vs. Males)	-0.020 (-0.111, 0.071)	0.669	-0.031 (-0.122, 0.060)	0.503
	PFOA × Sex (Interaction term)	-0.136 (-0.429, 0.158)	0.363	-0.140 (-0.437, 0.156)	0.350
T/E_2	PFOA	-0.160 (-0.426, 0.105)	0.235	-0.067 (-0.338, 0.204)	0.625
	Females (vs. Males)	-0.086 (-0.186, 0.013)	0.088	-0.070 (-0.166, 0.027)	0.154
	PFOA × Sex (Interaction term)	0.172 (-0.149, 0.493)	0.290	0.133 (-0.180, 0.446)	0.402

Associations between maternal PFOS or PFOA levels and infant sex on sex hormone levels were evaluated using multiple linear regression models.

[a] Crude: Non-adjusted.

[b] Adjusted: Adjusted for maternal age (years; continuous), maternal smoking during the third

trimester (yes/no), maternal alcohol consumption during pregnancy (yes/no), annual household income (< 5/≥ 5 million Japanese yen), parity (primipara/multipara), maternal blood sampling periods (during pregnancy or after birth), and infant birth weight (grams; continuous).

β (95% CI) represents change (95% confidence intervals) in $\log_{10}$-transformed A-dione (ng/mL), T (pg/mL), E_2 (ng/mL), or DHEA/A-dione levels for each 10-fold PFOS or PFOA level (ng/mL).

PFAS-sex interaction term was defined as "$\log_{10}$-transformed PFOS or PFOA levels (continuous) * sex (0 = males and 1 = females)".

Supplementary Table 6. Sensitivity analysis of associations between PFOS levels in maternal sera during pregnancy and female infant genotypes *CYP17A1* (rs743572) on sex hormone levels in cord sera among pregnant women and female infant pairs for which maternal blood samples were obtained before delivery

		Females (n = 88)			
		Crude[a]		Adjusted[b]	
Outcome	Exposure/Genotype	β (95% CI)	p value	β (95% CI)	p value
P_4	PFOS (ng/mL)	-0.410 (-0.955, 0.136)	0.139	-0.442 (-0.999, 0.115)	0.118
(ng/mL)	*CYP17A1* (rs743572)-AG/GG (vs. AA)	0.014 (-0.504, 0.533)	0.956	0.049 (-0.484, 0.581)	0.856
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	-0.165 (-0.856, 0.526)	0.636	-0.212 (-0.916, 0.493)	0.551
DHEA	PFOS (ng/mL)	0.410 (-0.116, 0.936)	0.125	0.409 (-0.139, 0.957)	0.142
(ng/mL)	*CYP17A1* (rs743572)-AG/GG (vs. AA)	0.059 (-0.441, 0.560)	0.815	0.008 (-0.515, 0.532)	0.975
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	0.010 (-0.656, 0.677)	0.976	0.066 (-0.627, 0.759)	0.851
A-dione	PFOS (ng/mL)	0.368 (0.020, 0.716)	0.039	0.388 (0.028, 0.748)	0.035
(ng/mL)	*CYP17A1* (rs743572)-AG/GG (vs. AA)	0.501 (0.170, 0.832)	0.003	0.484 (0.140, 0.828)	0.006
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	-0.768 (-1.210, -0.327)	0.001	-0.742 (-1.198, -0.287)	0.002
DHEA/A-dione	PFOS (ng/mL)	0.042 (-0.587, 0.670)	0.895	0.020 (-0.633, 0.674)	0.951
	CYP17A1 (rs743572)-AG/GG (vs. AA)	-0.442 (-1.040, 0.156)	0.145	-0.476 (-1.100, 0.149)	0.134
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	0.779 (-0.018, 1.575)	0.055	0.808 (-0.019, 1.635)	0.055
T	PFOS (ng/mL)	0.622 (0.171, 1.072)	0.007	0.639 (0.172, 1.107)	0.008
(pg/mL)	*CYP17A1* (rs743572)-AG/GG (vs. AA)	0.774 (0.345, 1.204)	0.001	0.750 (0.304, 1.197)	0.001
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	-1.181 (-1.753, -0.610)	<0.001	-1.147 (-1.738, -0.556)	<0.001
E_2	PFOS (ng/mL)	0.386 (0.031, 0.742)	0.034	0.377 (0.017, 0.738)	0.041
(ng/mL)	*CYP17A1* (rs743572)-AG/GG (vs. AA)	0.318 (-0.020, 0.657)	0.065	0.324 (-0.021, 0.668)	0.065
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	-0.527 (-0.978, -0.076)	0.023	-0.525 (-0.981, -0.069)	0.025
T/E_2	PFOS (ng/mL)	0.235 (-0.206, 0.677)	0.292	0.262 (-0.178, 0.703)	0.240
	CYP17A1 (rs743572)-AG/GG (vs. AA)	0.456 (0.036, 0.876)	0.034	0.426 (0.005, 0.848)	0.047
	PFOS × *CYP17A1* (rs743572)-AG/GG (Interaction term)	-0.654 (-1.214, -0.095)	0.022	-0.622 (-1.180, -0.064)	0.029

Associations between maternal PFOS levels and infant genotypes on sex hormone levels were evaluated using multiple linear regression models.

[a] Crude: Non-adjusted.

[b] Adjusted: Adjusted for maternal age (years; continuous), maternal smoking during the third trimester (yes/no), maternal alcohol consumption during pregnancy (yes/no), annual household income (< 5/≥ 5 million Japanese yen), parity (primipara/multipara), infant sex (male/female; all participants), maternal blood sampling periods (during pregnancy or after birth), and infant birth weight (grams; continuous).

β (95% CI) represents change (95% confidence intervals) in $\log_{10}$-transformed A-dione (ng/mL), T (pg/mL), E_2 (ng/mL), or DHEA/A-dione levels for each 10-fold PFOS level (ng/mL).

PFOS-*CYP17A1* (rs743572) interaction term was defined as "$\log_{10}$-transformed PFOS levels (continuous) * genotype (0 = AA and 1 =AG/GG)".

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Supplementary Table 7. Sensitivity analysis of PFOS levels in maternal sera and sex steroid hormone levels in cord sera stratified by female infant genotypes *CYP17A1* (rs743572) among pregnant women and female infant pairs for which maternal blood samples were obtained before delivery

		Exposure: PFOS (ng/mL)			
		Females (n = 88)			
	CYP17A1 (rs743572)	Crude[a]		Adjusted[b]	
Outcome	Infant genotype	β (95% CI)	p value	β (95% CI)	p value
P_4 (ng/mL)	AA	-0.410 (-0.996, 0.177)	0.163	-0.445 (-1.046, 0.157)	0.139
	AG/GG	-0.575 (-0.995, -0.155)	0.008	-0.589 (-1.052, -0.126)	0.014
DHEA (ng/mL)	AA	0.410 (-0.100, 0.920)	0.110	0.389 (-0.129, 0.907)	0.133
	AG/GG	0.420 (-0.003, 0.843)	0.052	0.408 (-0.069, 0.885)	0.092
A-dione (ng/mL)	AA	0.368 (-0.086, 0.822)	0.108	0.396 (-0.103, 0.894)	0.113
	AG/GG	-0.401 (-0.637, -0.164)	0.001	-0.378 (-0.643, -0.112)	0.006
DHEA/A-dione	AA	0.042 (-0.505, 0.589)	0.876	-0.007 (-0.600, 0.586)	0.981
	AG/GG	0.821 (0.298, 1.343)	0.003	0.786 (0.200, 1.371)	0.010
T (pg/mL)	AA	0.622 (0.085, 1.158)	0.025	0.678 (0.074, 1.281)	0.030
	AG/GG	-0.560 (-0.889, -0.231)	0.001	-0.483 (-0.853, -0.112)	0.012
E_2 (ng/mL)	AA	0.386 (-0.055, 0.828)	0.084	0.353 (-0.176, 0.881)	0.178
	AG/GG	-0.140 (-0.392, 0.111)	0.269	-0.137 (-0.406, 0.131)	0.309
T/E_2	AA	0.235 (-0.230, 0.701)	0.308	0.325 (-0.156, 0.807)	0.174
	AG/GG	-0.419 (-0.763, -0.076)	0.018	-0.345 (-0.729, 0.038)	0.077

Associations between maternal PFOS levels and sex hormone levels were evaluated using multiple linear regression models.

[a] Crude: Non-adjusted.

[b] Adjusted: Adjusted for maternal age (years; continuous), maternal smoking during the third trimester (yes/no), maternal alcohol consumption during pregnancy (yes/no), annual household income (< 5/≥ 5 million Japanese yen), parity (primipara/multipara), infant sex (male/female; all participants), maternal blood sampling periods (during pregnancy or after birth), and infant birth weight (grams; continuous).

β (95% CI) represents change (95% confidence intervals) in $\log_{10}$-transformed P_4 (ng/mL), DHEA (ng/mL), A-dione (ng/mL), DHEA/A-dione, T (pg/mL), E_2 (ng/mL), or T/E2 levels per 10-fold increase in the maternal PFOS levels.

Supplementary Table 8. Association of infant genotype *CYP17A1* (rs743572) with PFOS and PFOA levels in maternal blood and sex hormone levels in cord blood

	Infant genotype *CYP17A1* (rs743572)		
	AA	AG/GG	*p* value
PFOS (ng/mL)	5.5 (3.9, 7.3)	4.8 (3.2, 6.7)	0.145
PFOA (ng/mL)	1.4 (0.9, 2.3)	1.4 (0.9, 1.8)	0.279
P_4 (ng/mL)	236.7 (183.2, 306.0)	207.9 (170.6, 272.5)	0.068
DHEA (ng/mL)	2.1 (1.7, 3.2)	2.3 (1.9, 3.0)	0.345
A-dione (ng/mL)	0.44 (0.36, 0.61)	0.45 (0.36, 0.57)	0.644
DHEA/A-dione	5.0 (3.8, 6.1)	4.7 (3.7, 5.9)	0.780
T (pg/mL)	85.4 (57.8, 109.2)	84.2 (60.5, 111.7)	0.683
E_2 (ng/mL)	5.4 (3.6, 7.4)	4.7 (3.3, 7.1)	0.396
T/E_2	15.1 (11.7, 21.8)	18.0 (12.6, 22.8)	0.155

Median (inter-quartile range).

Mann-Whitney's *U*-test.