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1 Associations between maternal mono-(2-ethylhexyl) phthalate levels, nuclear receptor gene  
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#### 4 **Highlights**

5 ● Mono-(2-ethylhexyl) phthalate was associated with fatty acids.

6 ● Genotype *PPARGC1A* (rs8192678) affected oleic acid level.

7 ● Genotype *LXRβ* (rs2203044) affected linoleic acid level.

8 ● Gene-environment interaction persisted after adjusting for perfluorooctanesulfonate.

9

#### 10 **Abstract**

11 We assessed how the interaction between mono-(2-ethylhexyl) phthalate (MEHP) in maternal  
12 sera and the maternal genotypes associated with nuclear receptors affect fatty acid levels in a  
13 prospective birth cohort study of pregnant Japanese individuals ( $n = 437$ ) recruited in Sapporo  
14 between 2002 and 2005. We analyzed MEHP and fatty acids using gas chromatography-mass  
15 spectrometry. Thirteen single nucleotide polymorphisms of peroxisome proliferator-activated  
16 receptor (PPAR) alpha, PPAR gamma (*PPARG*), PPARG coactivator 1A (*PPARGC1A*), PPAR  
17 delta, constitutive androstane receptor, liver X receptor (LXR) alpha, and LXR beta (*LXRβ*)  
18 were analyzed using real-time PCR. Multiple linear regression models were used to confirm the

1 influence of log<sub>10</sub>-transformed MEHP levels and maternal genotypes on log<sub>10</sub>-transformed fatty  
2 acid levels. When the effects of the interaction between MEHP levels and the maternal  
3 *PPARGCIA* (rs8192678) genotype on oleic acid levels were evaluated, the estimated changes  
4 (95% confidence intervals) in oleic acid levels against MEHP levels, maternal *PPARGCIA*  
5 (rs8192678)-GA/AA genotype, and the interaction between them showed a mean reduction of  
6 0.200 (0.079, 0.322), mean reduction of 0.141 (0.000, 0.283), and mean increase of 0.145  
7 (0.010, 0.281), respectively, after adjusting for the perfluorooctanesulfonate level. The effects of  
8 the interaction between MEHP levels and maternal *LXRB* (rs2303044) genotype on linoleic acid  
9 levels was also significant ( $p_{int} = 0.010$ ). In conclusion, the interaction between MEHP and the  
10 maternal genotypes *PPARGCIA* (rs8192678) and *LXRB* (rs2303044) decreased fatty acid levels.  
11 Further, the interaction between MEHP and *PPARGCIA* (rs8192678) may have a greater effect  
12 on fatty acid levels than the interaction between PFOS and *PPARGCIA*.

13

#### 14 **Keywords**

15 Mono-(2-ethylhexyl) phthalate; Pregnancy; Genotype; Peroxisome proliferator-activated  
16 receptor coactivator 1A; Liver X receptor beta; Fatty acid

17

#### 18 **Abbreviations**

- 1 ABC, ATP-binding cassette
- 2 ABCB1, ATP-binding cassette, sub-family B, member 1
- 3 ABCC2, ATP-binding cassette, sub-family C, member 2
- 4 BMI, body mass index
- 5 CAR, constitutive androstane receptor
- 6 CI, confidence interval
- 7 DEHP, di-(2-ethylhexyl) phthalate
- 8 ESR1, estrogen receptor 1
- 9 GC/MS, gas chromatography-mass spectrometry
- 10 HWE, Hardy-Weinberg equilibrium
- 11 LC/MS/MS, liquid chromatography-tandem mass spectrometry
- 12 LD, linkage disequilibrium
- 13 LOD, limit of detection
- 14 LXR, liver X receptor
- 15 LXRA, liver X receptor alpha
- 16 LXRβ, liver X receptor beta
- 17 MDR1, multiple drug resistance 1
- 18 MEHP, mono-(2-ethylhexyl) phthalate

- 1 MRP2, multidrug resistance-associated protein 2
- 2 NCBI, National Center for Biological Information
- 3 PCR, polymerase chain reaction
- 4 PFAS, perfluoroalkyl substance
- 5 PON2, paraoxonase-2
- 6 PFOA, perfluorooctanoic acid
- 7 PFOS, perfluorooctanesulfonate
- 8 PPAR, peroxisome proliferator-activated receptor
- 9 PPARA, peroxisome proliferator-activated receptor alpha
- 10 PPARD, peroxisome proliferator-activated receptor delta
- 11 PPARG, peroxisome proliferator-activated receptor gamma
- 12 PPARGC1A, peroxisome proliferator-activated receptor gamma coactivator 1A
- 13 PVC, polyvinyl chloride
- 14 SD, standard deviation
- 15 SNP, single nucleotide polymorphism
- 16 SREBP1c, sterol response element-binding protein 1c
- 17 TCA cycle, tricarboxylic acid cycle
- 18 2D:4D, ratio of the lengths of the second and fourth digits

1

2

### 3 **1. Introduction**

4 Polyvinyl chloride (PVC) is a plastic material that exist in both hard and soft forms. It has a wide  
5 range of applications and is a common component of items such as shoes, bags, and industrial  
6 products. PVC is synthesized by the addition polymerization of vinyl chloride monomers, with  
7 di-(2-ethylhexyl) phthalate (DEHP) is added as a plasticizer to maintain the flexibility of PVC.  
8 The elution of DEHP from PVC has been previously demonstrated, and lipase has been shown to  
9 metabolize DEHP to mono-(2-ethylhexyl) phthalate (MEHP).

10         The effects of non-intentional exposure to DEHP and MEHP have been investigated.  
11 Previous studies have evaluated MEHP levels in the blood of non-pregnant female individuals.  
12 Specifically regarding the MEHP levels in venous blood, a Korean study reported mean MEHP  
13 levels of 15.8 ng/mL in a cohort with mean age of 34.8 years [1], an Italian study reported median  
14 MEHP levels of 8.3 ng/mL in a cohort with age range of 18-40 years [2], and another Italian study  
15 reported median MEHP levels of 580 ng/mL in a cohort with mean age of 37.8 years [3]. Some  
16 studies have also evaluated MEHP levels the blood of pregnant individuals, showing median  
17 MEHP levels in the range of 2.4-520 ng/mL in maternal or cord blood [4-10]. Thus, MEHP has  
18 been detected in both non-pregnant and pregnant female individuals. In human studies, changes

1 in maternal phthalate levels during pregnancy have been associated with alterations in the levels  
2 of sex hormones such as testosterone [11] and progesterone [12]. Although the secretion of many  
3 hormones changes during gestation [13], few data on the comparison of phthalate levels between  
4 non-pregnant and pregnant female individuals are currently available.

5 Fatty acid levels in pregnant individuals are closely associated with maternal hormone  
6 levels. The placenta contributes to maintaining pregnancy by secreting progesterone and estradiol  
7 after 10-12 weeks of pregnancy and the levels of these hormone continue to rise until delivery  
8 [14]. High maternal progesterone levels during pregnancy are associated with increased  
9 docosahexaenoic acid levels [15]. Progesterone and estradiol are the hormones whose levels are  
10 altered during pregnancy and other many hormones may be involved in adjusting tissue fatty acid  
11 composition. These findings indicate that investigating both the adverse health effects of exposure  
12 to DEHP and MEHP and maternal fatty acid levels during pregnancy is important for maintaining  
13 the health of pregnant individuals.

14 Epidemiological studies have shown that maternal exposure to MEHP during pregnancy  
15 leads to the development of adverse health effects in the offspring. Increased maternal MEHP  
16 levels are associated with reduced levels of triglycerides and fatty acids, such as palmitic acid,  
17 oleic acid, linoleic acid, and  $\alpha$ -linolenic acid, though no such association exists between maternal  
18 MEHP levels and the levels of stearic acid, palmitoleic acid, arachidonic acid, eicosapentaenoic

1 acid, and docosahexaenoic acid [16]. Increased maternal MEHP levels during pregnancy are also  
2 associated with reduced body mass index (BMI) in children aged 4 to 7 years [17,18]. Analysis  
3 of the association between prenatal phthalate exposure to MEHP, mono-(2-ethyl-5-hydroxyhexyl)  
4 phthalate, and mono-(2-ethyl-5-oxohexyl) phthalate and child BMI at 4 and 6 years of age [19]  
5 has produced inconsistent results. This discrepancy may be the result of differences in MEHP  
6 levels and maternal genetic factors between individuals. MEHP and DEHP induce receptor  
7 activation by binding to receptors [20-26]. Subsequently, certain biological signals are amplified  
8 or mitigated following receptor activation [20-31]. It is, therefore, likely that maternal MEHP  
9 interacts with the maternal nuclear receptors involved in maternal lipid homeostasis [20-31].

10 DEHP binds to peroxisome proliferator-activated receptor (PPAR) alpha (PPARA) [24],  
11 PPAR delta (PPARD) [24], and constitutive androstane receptor (CAR) [22]. MEHP binds to  
12 PPARA [26], PPAR gamma (PPARG) [20,24,26], CAR [21], and liver X receptor (LXR) alpha  
13 (LXRA) [23,25]. Since LXRA and LXR beta (LXRB) share a high degree of homology [27],  
14 suggesting that DEHP and MEHP bind to LXRB, as well, though only a few studies have reported  
15 this phenomenon (see also Fig. 1 of the relationship between phthalates, receptors, and fatty acid  
16 [20-31]). Genetic and environmental factors may also play a role in regulating fatty acid levels  
17 [32]. Previous studies have identified gene-environment interactions that support the hypothesis  
18 that environmental factors, such as exposure to MEHP, and an individual's genotype, particularly

1 that relating to nuclear receptors, together determine their health outcomes [33-39]. Thus, it is  
2 important to investigate the gene-environment interactions that affect maternal fatty acid levels.

3       Increased maternal MEHP levels during pregnancy have been associated with increased  
4 risk of low birth weight associated with the paraoxonase-2 (*PON2*; Ala148Gly) genotype [38] and  
5 with a decreased ratio of the lengths of the second and fourth digits (2D:4D) in the offspring. The  
6 ratio, calculated in offspring aged 7 years, is considered an index of prenatal exposure to sex  
7 hormones due to its association with the child estrogen receptor 1 (*ESR1*; rs2077647) genotype  
8 [37]. However, no association between maternal MEHP levels and genotypes related to orphan  
9 receptors and blood fatty acid levels has yet been reported. We previously reported an association  
10 between maternal MEHP levels and the levels of some fatty acids in maternal blood [16]. We also  
11 showed the effects of the gene-environment interactions between prenatal perfluoroalkyl  
12 substance (PFAS) levels and maternal PPARG coactivator 1A (*PPARGC1A*; rs8198678) and  
13 *PPARD* (rs1053049 and rs2267668) genotypes on maternal fatty acid levels [36].

14       PFASs are called "forever chemicals", and their environmental pollution has increasingly  
15 become a societal concern. PFASs, as well as MEHP, are also known to activate PPARs, CAR,  
16 and LXRs [40]. Since both PFASs and MEHP activate these nuclear receptors, it is possible that  
17 exposure to PFASs during pregnancy modifies the association between maternal MEHP and the  
18 genotypes associated with these nuclear receptors, thereby affecting maternal fatty acid levels

1 during pregnancy. We hypothesized that the adverse effects associated with the reduction in  
2 maternal fatty acid levels due to PFAS exposure is partially compounded by the reduction in  
3 maternal fatty acid levels due to MEHP exposure during pregnancy, thereby causing fetal growth  
4 restriction. We deemed it necessary to consider the PFAS-mediated change as having a similar  
5 biological mechanism to the MEHP-mediated change when investigating this association.

6 Similar to previously conducted follow-up studies [16,36], this study aimed to examine the  
7 effects of the association between MEHP levels and 13 genetic polymorphisms related in the  
8 *PPARs*, *CAR*, and *LXRs* genes on fatty acid levels in pregnant Japanese individuals.

9

## 10 **2. Materials and methods**

### 11 ***2.1. Study participants***

12 This prospective birth cohort study was based on the Hokkaido Study on Environment and  
13 Children's Health (Sapporo cohort). The study protocol has been described previously [41].  
14 Briefly, from July 2002 to October 2005, pregnant Japanese women ( $n = 514$ ) were recruited from  
15 a local obstetrics and gynecology hospital in Sapporo City. Of these, 10 participants withdrew  
16 from the study. Of the remaining subjects, 437 participants provided complete data on the levels  
17 of MEHP, PFOS, PFOA, fatty acid, and maternal genotypes.

18

1    **2.2. Ethical approval**

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

7

8    **2.3. Data collection**

9    Each participant completed a self-administered questionnaire at enrollment regarding age, height  
10    before pregnancy, weight before pregnancy, annual household income, smoking in the 3<sup>rd</sup>  
11    trimester, and alcohol consumption during pregnancy. Maternal records were also obtained to  
12    collect parity information.

13

14    **2.4. Measurement of sera MEHP and fatty acid levels in maternal samples**

15    A 40 mL blood sample was collected from the maternal peripheral vein in the 3<sup>rd</sup> trimester. All  
16    samples were stored at -80 °C until analysis. In 491 maternal blood samples, fatty acid levels were  
17    measured using gas chromatography-mass spectrometry (GC/MS) at Nagoya University as  
18    described previously [16,36]. The samples of the remaining participants were not analyzed

1 because of unavailability or insufficient sample volume. Of the 491 blood samples, 307 were  
2 collected during pregnancy, and 184 were obtained after delivery owing to anemia during  
3 pregnancy. Nine fatty acids were targeted for measurement including palmitic acid, palmitoleic  
4 acid, stearic acid, oleic acid, linoleic acid,  $\alpha$ -linolenic acid, arachidonic acid, eicosapentaenoic  
5 acid, and docosahexaenoic acid. The detection limits were set as 278.34 pg/mL for MEHP, 2.4  
6  $\mu\text{g/mL}$  for palmitic acid, 0.069  $\mu\text{g/mL}$  for palmitoleic acid, 1.3  $\mu\text{g/mL}$  for stearic acid, 3.6  $\mu\text{g/mL}$   
7 for oleic acid, and 2.0  $\mu\text{g/mL}$  for the other FAs. The detection rates for all MEHP and fatty acids  
8 were more than 99.0% except for eicosapentaenoic acid (detection limit: 97.8%). Non-fasting  
9 blood triglyceride levels were measured using triglyceride E-Test Wako Kits (Wako, Osaka,  
10 Japan) after lipid extraction according to the methods described by Folch et al. [42].

11

12 ***2.5. Measurement of PFAS (perfluorooctanesulfonate [PFOS] and perfluorooctanoic acid***  
13 ***[PFOA]) levels in maternal sera***

14 A 40 mL blood sample was collected from the maternal peripheral vein in the 3<sup>rd</sup> trimester. All  
15 samples were stored at -80 °C until analysis. PFOS and PFOA levels were measured in 447  
16 maternal blood samples using liquid chromatography-tandem mass spectrometry (LC/MS/MS).  
17 The measurement protocol has been described previously [36,43]. The samples of the remaining  
18 participants were not analyzed because of unavailability or insufficient sample volume. Of the

1 447 blood samples, 228 were collected during pregnancy and 159 were obtained after delivery  
2 owing to anemia during pregnancy. All samples exceeded the limit of detection (LOD; 0.50  
3 ng/mL) for PFOS. However, 16 (5.9%) samples had PFOA levels below the LOD (0.50 ng/mL),  
4 and these cases were assigned a value of 0.25 ng/mL (50% of LOD).

5

## 6 **2.6. Assessment of maternal genotypes**

7 We analyzed the genotypes of 494 maternal blood samples. The remaining samples were not  
8 analyzed because of unavailability or insufficient blood volume. Maternal blood samples were  
9 collected when participants gave birth, and 400  $\mu$ L of each sample was used to isolate and purify  
10 genomic DNA using a QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) or a  
11 Maxwell 16 DNA Purification Kit (Promega, Madison, WI, USA), according to the  
12 manufacturer's instructions [44]. First, we focused three genes of the *PPARs*, *CAR*, and *LXRs*,  
13 from orphan receptors that are expected to be activated by MEHP and affected by fatty acid levels.  
14 Next, using the single nucleotide polymorphism (SNP) database of the National Center for  
15 Biological Information (NCBI), we evaluated 13 genetic polymorphisms that were located in  
16 potentially functional regions (mainly promotor and coding regions) and were associated with  
17 susceptibilities to diseases such as cancer, nonalcohol fatty acid disease, type 2 diabetes mellitus,  
18 and obesity: *PPARA* (T>C, Val227Ala; dbSNP ID: rs1800234; G>A, dbSNP ID: rs135561),

1 *PPARD* (T>C, dbSNP ID: rs1053049; A>G, dbSNP ID: rs2267668), *PPARG* (C>T, His449His;  
2 dbSNP ID: rs3856806), *PPARGCIA* (C>T, Thr394Thr; dbSNP ID: rs2970847; G>A, dbSNP ID:  
3 rs8192678), *CAR* (T>C, Pro180Pro; dbSNP ID: rs2307424; A>G, dbSNP ID: rs2501873), *LXRA*  
4 (C>T, Ser99Ser; dbSNP ID: rs2279238), and *LXRB* (T>C, dbSNP ID: rs1405655; G>A, dbSNP  
5 ID: rs2303044; G>A; dbSNP ID: rs4802703) [45-55]. The evaluation was performed based on  
6 the analysis of high-throughput gene expression of preamplification, real-time polymerase chain  
7 reaction (PCR) performed with dynamic chips, and TaqMan gene expression measurements. The  
8 assessment protocol has been described previously [36]. Approximately 5% or more minor alleles  
9 among pregnant Japanese women are necessary to secure statistical powers for examining the  
10 adverse health outcome. All 13 genetic polymorphisms satisfied a minor allele frequency of > 5%.

11

## 12 **2.7. Statistical analyses**

13 Of the 437 participants, data of one (0.2%) and nine (2.1%) samples corresponding to parity and  
14 annual household income, respectively were missing. Using simple imputation, the participants  
15 were assigned to the parity group of multiparous and annual household income of < 5 million  
16 Japanese yen (the most frequent group). First, we examined the characteristics of the participants.  
17 Second, a chi-squared test was employed to test whether the frequency of genotype distribution  
18 conformed to the Hardy-Weinberg equilibrium (HWE). Third, a case-control study was conducted

1 with the case of a lower than median oleic acid level ( $n_{case} = 218$ ) and the control of a higher than  
2 median oleic acid level ( $n_{control} = 219$ ), and linkage disequilibrium (LD) was evaluated using  
3 linkage analyses. Fourth, MEHP, PFOS, PFOA, and fatty acid levels were  $\log_{10}$ -transformed  
4 before the following analyses because of their non-normal distribution. Multiple linear regression  
5 analyses were used to evaluate the association between MEHP and fatty acid levels in both crude  
6 and adjusted models. Maternal age, maternal smoking in the 3<sup>rd</sup> trimester, maternal alcohol  
7 consumption during pregnancy, parity, annual household income, and maternal blood sampling  
8 periods (up to this covariate, their covariates applied to both adjusted 1 and 2), and PFOS levels  
9 (adjusted only 2) were adjusted in the multiple linear regression models, except in the crude  
10 models. Fifth, multiple linear regression analyses were used to evaluate the interaction between  
11 MEHP and PFOS or PFOA levels with fatty acid levels in both the crude and adjusted (adjusted  
12 only 1) models. The covariates were the same as those used in the fourth analysis. Sixth, multiple  
13 linear regression analyses were used to evaluate the interaction between MEHP levels and  
14 maternal genotypes with fatty acid levels in both the crude and adjusted (both adjusted 1 and 2)  
15 models. The covariates were the same as those used in the fourth analysis. Seventh, multiple linear  
16 regression analyses were used to evaluate the association between MEHP and fatty acid levels in  
17 both the crude and adjusted (both adjusted 1 and 2) models after stratification based on maternal  
18 genotypes *PPARGC1A* (rs8192678) and *LXR1B* (rs2303044), because only the effects of the

1 interactions between MEHP levels and maternal genotype *PPARGC1A* (rs8192678) on oleic acid  
2 levels and the interactions between MEHP levels and maternal genotype *LXR1B* (rs2303044) with  
3 linoleic acid levels were statistically significant. The covariates were the same as those used in  
4 the fourth analysis.

5 Data were considered statistically significant at  $p < 0.05$ . All statistical analyses were  
6 performed using SPSS software version 26 (IBM Corp., Armonk, NY, USA), except for the  
7 linkage analyses. Linkage analyses were performed using Haploview 4.2 software (Broad  
8 Institute of Massachusetts Institute of Technology and Harvard, USA) [56].

9

### 10 **3. Results**

11 The demographic variables and genotype frequencies for participants in the Hokkaido study are  
12 summarized in Table 1. Mean maternal age (standard deviation; SD) was 30.2 (4.8) years. The  
13 frequencies of maternal smokers in the 3<sup>rd</sup> trimester, those reporting alcohol consumption during  
14 pregnancy, multiparous mothers, and mothers belonging to households with an annual income of  
15  $\geq 5$  million Japanese yen were 18.3%, 29.7%, 52.4%, and 28.8%, respectively. Of all participants,  
16 blood samples of 64.3% of the mothers were collected during pregnancy. Median levels (inter-  
17 quartile range) of MEHP, triglyceride, palmitic acid, palmitoleic acid, stearic acid, oleic acid,  
18 linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid

1 were 10.2 (5.9, 16.3) ng/mL, 84.5 (61.3, 117.8) mg/100-mL, 1,883.2 (1,558.1, 2,445.6) µg/mL,  
2 108.5 (78.5, 160.0) µg/mL, 530.2 (433.6, 635.2) µg/mL, 1,128.8 (882.8, 1,439.4) µg/mL, 714.2  
3 (500.1, 946.6) µg/mL, 10.1 (5.4, 15.3) µg/mL, 64.7 (44.5, 93.9) µg/mL, 8.5 (4.8, 14.0) µg/mL,  
4 and 25.0 (13.7, 39.0) µg/mL, respectively. The distribution of 12 SNPs satisfied HWE (chi-  
5 squared test: all  $p > 0.05$ ) in 437 participants, except for *PPARD* (rs1053049; chi-squared value =  
6 4.636;  $p = 0.031$ ). Moreover, no associations were observed between MEHP levels and maternal  
7 genotypes and between fatty acid levels and maternal genotypes (data not shown).

8 The LD plot of the 13 SNPs is shown in Fig. 2. The LD parameter values ( $D'$ ) for  
9 *PPARGCIA* (rs2970847 and rs8192678), *PPARD* (rs1053049 and rs2267668), and *LXRβ*  
10 (rs1405655, rs2303044, and rs4802703) were  $\geq 0.90$ . Except for these values,  $D'$  was less than  
11 0.90.

12 The effects of maternal MEHP levels on fatty acid levels are summarized in Table 2.  
13 Multiple linear regression analysis showed that MEHP levels were associated with lower levels  
14 of palmitic acid (mean reduction = 0.093 [95% confidence interval (CI): 0.035, 0.151]),  
15 palmitoleic acid (mean reduction = 0.115 [95% CI: 0.031, 0.200]), oleic acid (mean reduction =  
16 0.089 [95% CI: 0.025, 0.154]), linoleic acid (mean reduction = 0.140 [95% CI: 0.007, 0.272]),  
17 and linolenic acid (mean reduction = 0.163 [95% CI: 0.009, 0.317]) after adjusting for covariates  
18 including PFOS levels. Interestingly, for eicosapentanoic acid and docosahexaenoic acid levels,

1 we observed only a statistically significant interactions between MEHP and PFOS levels after  
2 adjusting for covariates ( $p$ -value for interaction [ $p_{int}$ ] = 0.034 for eicosapentanoic acid and 0.035  
3 for docosahexanoic acid; Supplementary Table 1). There was no statistically significant  
4 interaction between MEHP and PFOA levels with fatty acid levels (data not shown).

5 The combined effects of the interactions of MEHP levels and maternal *PPARGCIA*  
6 (rs8192678) genotype on fatty acid levels are presented in Table 3 (see also Fig. 3.A.). Multiple  
7 linear regression analysis showed a significant interaction between MEHP levels and maternal  
8 *PPARGCIA* (rs8192678) genotype on oleic acid levels after adjusting for covariates, including  
9 PFOS levels. When the effect of the interaction between MEHP levels and maternal *PPARGCIA*  
10 (rs8192678) genotype on oleic acid levels was analyzed, the estimated changes (95% CI) in oleic  
11 acid levels against an increase of one unit in MEHP levels, those against maternal *PPARGCIA*  
12 (rs8192678)-GA/AA genotype, and those against interaction between MEHP levels and maternal  
13 *PPARGCIA* (rs8192678)-GA/AA genotype showed a mean reduction of 0.200 (0.079, 0.322), a  
14 mean reduction of 0.141 (0.000, 0.283), and a mean increase of 0.145 (0.010, 0.281) ( $p_{int}$  = 0.036),  
15 respectively, after adjusting for covariates, including PFOS levels. The effects of MEHP levels on  
16 oleic acid levels stratified by maternal *PPARGCIA* (rs8192678) genotype were analyzed.  
17 Estimated changes (95% CI) in oleic acid levels against an increase of one unit in MEHP levels  
18 involved a mean reduction of 0.130 (-0.271, 0.010) in mothers with the GG genotype and a mean

1 reduction of 0.077 (0.004, 0.151) in mothers with the GA/AA genotype, after adjusting for the  
2 covariates, including PFOS levels (Supplementary Table 2).

3         The effects of combined interactions of MEHP levels and maternal *LXRB* (rs2303044)  
4 genotype on fatty acid levels are presented in Table 4 (see also Fig. 3.B.). Multiple linear  
5 regression analysis showed a significant effect of the interaction between MEHP levels and  
6 maternal *LXRB* (rs2303044) genotype on linoleic acid levels after adjusting for covariates,  
7 including PFOS levels. When the effects of the interactions between MEHP levels and maternal  
8 *LXRB* (rs2303044) genotype on linoleic acid levels were analyzed, the estimated changes (95%  
9 CI) in linoleic acid levels against an increase of one unit in MEHP levels, and those against the  
10 interaction between MEHP levels and maternal *LXRB* (rs2303044)-GA/AA genotype showed a  
11 mean reduction of 0.246 (0.092, 0.400), and a mean increase of 0.330 (0.079, 0.581) ( $p_{int} = 0.010$ ),  
12 respectively, after adjusting for the covariates, including PFOS levels. When the effects of MEHP  
13 levels on linoleic acid levels stratified by maternal *LXRB* (rs2303044) genotype were analyzed,  
14 the estimated changes (95% CI) in oleic acid levels against an increase of one unit in MEHP levels  
15 involved a mean reduction of 0.224 (0.052, 0.395) in mothers with the GG genotype and a mean  
16 increase of 0.071 (-0.124, 0.267) in mothers with the GA/AA genotype after adjusting for the  
17 covariates, including PFOS levels (Supplementary Table 3). No statistically significant interaction  
18 was observed between MEHP levels and 11 SNPs of *PPARA* (rs1800234 and rs135561), *PPARG*

1 (rs3856806), *PPARGCIA* (rs2970847), *PPARD* (rs1053049 and rs2267668), *CAR* (rs2307424 and  
2 rs2501873), *LXRA* (rs2279238), and *LXRB* (rs1405655 and rs4802703) with fatty acid levels (data  
3 not shown).

4

#### 5 **4. Discussion**

6 We examined the association between MEHP levels and fatty acid levels while considering the  
7 impact of PFOS levels in statistical analyses, since we found an association between the PFOS  
8 and fatty acid levels during pregnancy in our previous study [36], and the MEHP levels were  
9 correlated with the PFOS levels in this study (Spearman's  $\rho = 0.467$ ;  $p < 0.001$ ). Our results  
10 showed that the interactions between MEHP and the maternal *PPARGCIA* (rs8192678) genotypes,  
11 and that between MEHP and the maternal *LXRB* (rs2303044) genotype during pregnancy affected  
12 on the oleic acid and linoleic acid levels in maternal blood, respectively. The results of this study  
13 suggest that the prenatal gene-environment interactions MEHP and specific maternal genotypes  
14 modify the fatty acid levels in maternal blood. In a cross-sectional study across 184 countries, a  
15 decrease in the total long-chain omega-3 polyunsaturated fatty acid level in pregnant individuals  
16 (mean: 310 mg/day) was shown to be associated with an increased preterm birth rate [57]. In  
17 another study (mean linoleic acid: 19.07 ng/mL), infants of pregnant individuals with linoleic acid  
18 levels of 16.95-18.37 ng/mL in maternal plasma were increase of 11.0-g compared to pregnant

1 individuals with linoleic acid levels of 18.37-19.65 ng/mL [58]. In this study, the 0.224-unit  
2 reduction in  $\log_{10}$ -transformed linoleic acid levels per unit increase in  $\log_{10}$ -transformed MEHP  
3 levels in the maternal *LXRB* (rs2303044) GG genotype was approximately equal to a 1.7 ng/mL  
4 in linoleic acid levels. We speculate, based on previous study results, that the decrease in oleic  
5 acid and linoleic acid levels in this study corresponds to approximately a 10 g change in birth  
6 weight when proportional calculation is performed [58]. Reduction of 10-g is equal to reduction  
7 of 1/300 of the mean birth weight in Japan [59].

8         Some studies have evaluated MEHP levels in maternal or cord blood during pregnancy. The  
9 median MEHP levels in maternal blood have been reported as follows: 2.4 ng/mL at 37-42 weeks  
10 of pregnancy (Japanese study) [4], 3.69 ng/mL in the median 23<sup>rd</sup> week of pregnancy from 1997-  
11 2001 (Danish study) [5], 3.59 ng/mL in the mean 18<sup>th</sup> weeks of pregnancy from 2012-2014  
12 (Danish study) [5], 3.73 ng/mL during both weeks 18 and 34-36 of pregnancy (Australian study)  
13 [5], 6.14 ng/mL in the mean 12<sup>th</sup> week of pregnancy (Chinese study) [6], and 680 ng/mL in the  
14 mean 34.4<sup>th</sup> week of pregnancy (Italian study) [7]. Median MEHP levels in cord blood have been  
15 reported as follows: 3.02 ng/mL (Taiwanese study) [8], 130 ng/mL (Turkish study) [9], and 520  
16 ng/mL (Italian study) [10]. The MEHP levels in blood in this study were lower than those reported  
17 in the Italian [7,10], Turkish [9], and Chinese [6] studies but was higher than those reported in the  
18 Japanese [4], Taiwanese [8], Australian [5], and Danish [5] studies.

1           Although MEHP levels do not directly affect the PFAS levels in pregnant Japanese women,  
2           the interaction between MEHP and the maternal *PPARGCIA* (rs8192678) genotype affected oleic  
3           acid levels after adjusting for PFOS levels. In our previous study, the interaction between PFOS  
4           and the maternal *PPARGCIA* (rs8192678) genotype affected triglyceride, palmitic acid,  
5           palmitoleic acid, and oleic acid levels [36]. No effects of the interaction between MEHP and PFOS  
6           levels on triglyceride, palmitic acid, palmitoleic acid, and oleic acid levels, and correlation  
7           between MEHP and PFOS levels (Spearman's  $\rho = 0.467$ ;  $p < 0.001$ ), correlation between oleic  
8           acid and triglyceride (Spearman's  $\rho = 0.628$ ;  $p < 0.001$ ), palmitic acid (Spearman's  $\rho = 0.912$ ;  $p$   
9            $< 0.001$ ), and palmitoleic acid (Spearman's  $\rho = 0.751$ ;  $p < 0.001$ ) levels were observed in this  
10          study. This suggests that the interaction between MEHP and *PPARGCIA* (rs8192678) has a  
11          greater effect on fatty acid levels than the interaction between PFOS and *PPARGCIA* (rs8192678).  
12          Furthermore, it has been suggested that activation of PPAR by MEHP may increase  
13          xenobiotic metabolism [60].

14          In this study, two *PPARGCIA* genes (rs2970847 and rs8192678) showed LD. The  
15          *PPARGCIA* (rs8192678) GG genotype is associated with an increased risk of metabolic syndrome  
16          [61] and unstable angina [62], and individuals with this genotype show lower reductions in total  
17          cholesterol after consumption of a low-fat diet [63] than those with the GA/AA genotype.  
18          Therefore, we speculated that the rate of lipid metabolism of individuals with the *PPARGCIA*

1 (rs8192678) GG genotype was lower than that of individuals with the GA/AA genotype. We found  
2 that a decrease in the rate of lipid metabolism was associated with the effect of the interaction  
3 between MEHP and the maternal *PPARGCIA* (rs8192678) genotype on oleic acid levels. Oleic  
4 acid is secreted by astrocytes and used by neurons for the synthesis of phospholipids. Further, it  
5 is specifically incorporated into growth cones to promote axonal growth [64]. Hence, it may be  
6 that reduction in oleic acid levels affects the long-term suppression of brain differentiation.

7 We observed that the interaction between MEHP and the maternal *LXRB* (rs2303044)  
8 genotype affected linoleic acid levels after adjusting for PFOS levels. DEHP, the parent compound  
9 of MEHP, activates the LXR signaling pathway in mice [65]. Further, MEHP exposure during  
10 pregnancy leads to upregulation of *LXRA* mRNA expression in human testis somatic cells [20].  
11 Activation of *LXRA* and *LXRB* is associated with increased transcription of the sterol response  
12 element-binding protein 1c (SREBP1c) and of the downstream effectors involved in lipid  
13 synthesis in mice [66]. Hence, we speculated that increased MEHP exposure precipitated a  
14 decrease in linoleic acid levels via its interaction with maternal *LXRB*.

15 Information on the *LXRB* (rs2303044 and rs4802703) genes and polymorphisms is limited.  
16 The *LXRB* (rs2303044 and rs4802703) genotypes are not associated with an increased risk of type  
17 2 diabetes mellitus [53]. In this study, three *LXRBs* (rs1405655, rs2303044, and rs4802703)  
18 showed LD. In a previous study, six *LXRBs* (rs1405655 [intron 7], rs4802703 [intron 8],

1 rs2303045 [intron 7], rs17373080 [5' near gene], rs28514894 [intron 4], and rs41432149 [intron  
2 6]) satisfied the LD [53] criteria. The LD of the *LXRB* (rs1403655 and rs4802703) genotypes  
3 observed in this study was similar to that in a previous study [53].

4 Individuals with the major homozygous genotype (TT) of *LXRB* (rs1405655) have a 1.75-  
5 fold higher risk of developing Alzheimer's disease than those with the TC/CC genotype [67]. LXR  
6 upregulates the expression of xenobiotic transport proteins, such as multiple drug resistance 1  
7 (MDR1) (ATP-binding cassette [ABC], sub-family B, member 1; ABCB1) [68] and multidrug  
8 resistance-associated protein 2 (MRP2) (ABC, subfamily C, member 2; ABCC2) [69]. As  
9 increased linoleic acid levels were associated with increased protein levels of ABC transporters,  
10 such as MDR1 and MRP2 [70], we speculated that individuals with a major homozygous  
11 genotype (GG) of *LXRB* (rs2303044) had lower activation of the ABC transporter than the GA/AA  
12 genotype. As the ABC transporter is an excretion transporter able to carry molecules with a wide  
13 variety of chemical structures to the outside of the cell. Thus, decreased activation of the ABC  
14 transporter may lead to long-term adverse health effects due to the accumulation of chemical  
15 substances within the cells. Therefore, the interaction between MEHP levels and the maternal  
16 *LXRB* (rs2303044) genotype could precipitate a reduction in linoleic acid levels.

17 We observed that the specific genotypes *PPARA* (rs1800234) TT, *PPARG* (rs3856806) GG,  
18 *PPARD* (rs1053049) TT, *PPARGC1A* (rs2970847), *CAR* (rs2303044) AA, and *LXRA* (rs2279238)

1 AA were associated with MEHP and fatty acid levels (data not shown). The association between  
2 many of these genotypes with PFOS and fatty acid levels was also observed in our previous study  
3 [15]. No statistically significant associations between MEHP and fatty acid levels were found  
4 after adjusting for PFOS levels in this study (data not shown). Our results suggest that the  
5 associations between MEHP and fatty acid levels are largely modified by PFOS levels.

6 To the best of our knowledge, four previous epidemiological studies have examined the  
7 association between phthalate exposure and fatty acid levels [71-74]. The levels of the hydrophilic  
8 metabolites of phthalate diesters, such as MEHP, in the serum are 1/30 to 1/100-fold lower than  
9 those in the urine [75]. The National Health and Nutrition Examination Survey in the United  
10 States demonstrated a negative correlation between MEHP levels (Median: 1.4 ng/mL [equivalent  
11 to 0.15-0.50 nmol/mL in blood]; inter-quartile range: 0.6-3.5 ng/mL [equivalent to 0.13-1.26  
12 nmol/mL in blood]) and arachidonic acid levels [80], which is consistent with the results of our  
13 study, though unlike in our study, no such negative correlation was found with other fatty acids.  
14 The differences seen in these results may be caused to the differences between populations (the  
15 general population in the United States versus pregnant individuals in Japan) and sample types  
16 (urine versus blood). The remaining previous studies [71,73,74] referenced here did not describe  
17 the effects of MEHP exposure on fatty acid levels.

18 The strength of this study is that we accurately measured blood MEHP and fatty acid levels

1 using GC/MS. The present study has several limitations. First, multiple comparisons were made.  
2 The LDs of 13 genetic polymorphisms were first investigated to avoid the problem associated  
3 with multiple comparisons as much as possible. Since LD was confirmed at three sites, the  
4 associations between the MEHP levels, genotypes, and fatty acid levels were examined for 10  
5 genetic polymorphisms. However, no significant association was found after Bonferroni  
6 correction. It is necessary to re-examine these association with larger sample sizes to confirm  
7 their validity. Second, the Hokkaido Study on Environment and Children's Health is based on  
8 healthy pregnant individuals enrolled at a gynecology and obstetrics hospital in Sapporo, Japan.  
9 The study primarily comprised Japanese participants, and our findings might, thus, not apply to  
10 other populations due to the differences in genotype frequency. Third, we did not gather  
11 information on the medical interventions with pregnant individuals that use DEHP-containing  
12 plastics, though DEHP is known to leach into blood with the use of PVC-plasticizer DEHP in  
13 medical products [76]. However, we confirmed that the MEHP levels were not significantly  
14 different between the blood samples taken during pregnancy (median [inter-quartile range]: 10.2  
15 [6.0-15.2] ng/mL) and those taken after birth (median [inter-quartile range]: 10.1 [5.8-17.5]  
16 ng/mL) by the Mann-Whitney's *U*-test ( $p = 0.632$ ). Fourth, we did not adjust for characteristic  
17 gestational hormonal changes that take place during pregnancy. There were significant  
18 correlations between gestational blood sampling period of pregnant individuals who gave blood

1 samples during pregnancy and the levels of some fatty acids, such as triglyceride (Spearman's  $\rho$   
2 = 0.229;  $p < 0.001$ ), palmitic acid (Spearman's  $\rho = 0.165$ ;  $p = 0.004$ ), palmitoleic acid (Spearman's  
3  $\rho = 0.203$ ;  $p < 0.001$ ), and oleic acid (Spearman's  $\rho = 0.226$ ;  $p < 0.001$ ). The blood sampling  
4 period for analyzing fatty acids is the same as the blood sampling period for analyzing phthalates  
5 which is associated with the levels of some sex hormone levels in other previous studies.  
6 Adjusting each blood sampling period may be the results in over-adjustment. The adjusting factor  
7 as hormonal changes that are characteristic of the gestational period would be replaced by the  
8 gestational blood sampling period. Hence, we believe that the hormonal changes characteristics  
9 to the gestational period were considered in this study. Fifth, the effect of the interaction between  
10 MEHP and maternal genotypes on fatty acid levels might not have been correctly evaluated due  
11 to the limited sample size. Thus, in the near future, we would like to re-examine this effect using  
12 prospective birth cohort data with a larger sample size.

13

## 14 **5. Conclusion**

15 Our study demonstrated that the maternal genotype *PPARGCIA* (rs8192678) modified the  
16 association between MEHP and oleic acid levels, and maternal genotype *LXRB* (rs2303044)  
17 modified the association between MEHP and linoleic acid levels. Further, the interaction between  
18 MEHP and *PPARGCIA* (rs8192678) likely has a greater effect on fatty acid levels than the

1 interaction between PFOS and *PPARGCIA* (rs8192678). The results of this study suggest that  
2 certain genotypes may be associated with a preventive function involved in remaining adequate  
3 fatty acid levels by maintaining low DEHP levels.

4

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8

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16

#### 17 **Conflicts of interest**

18 The authors declare no conflicts of interest.

1

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## Figure legends

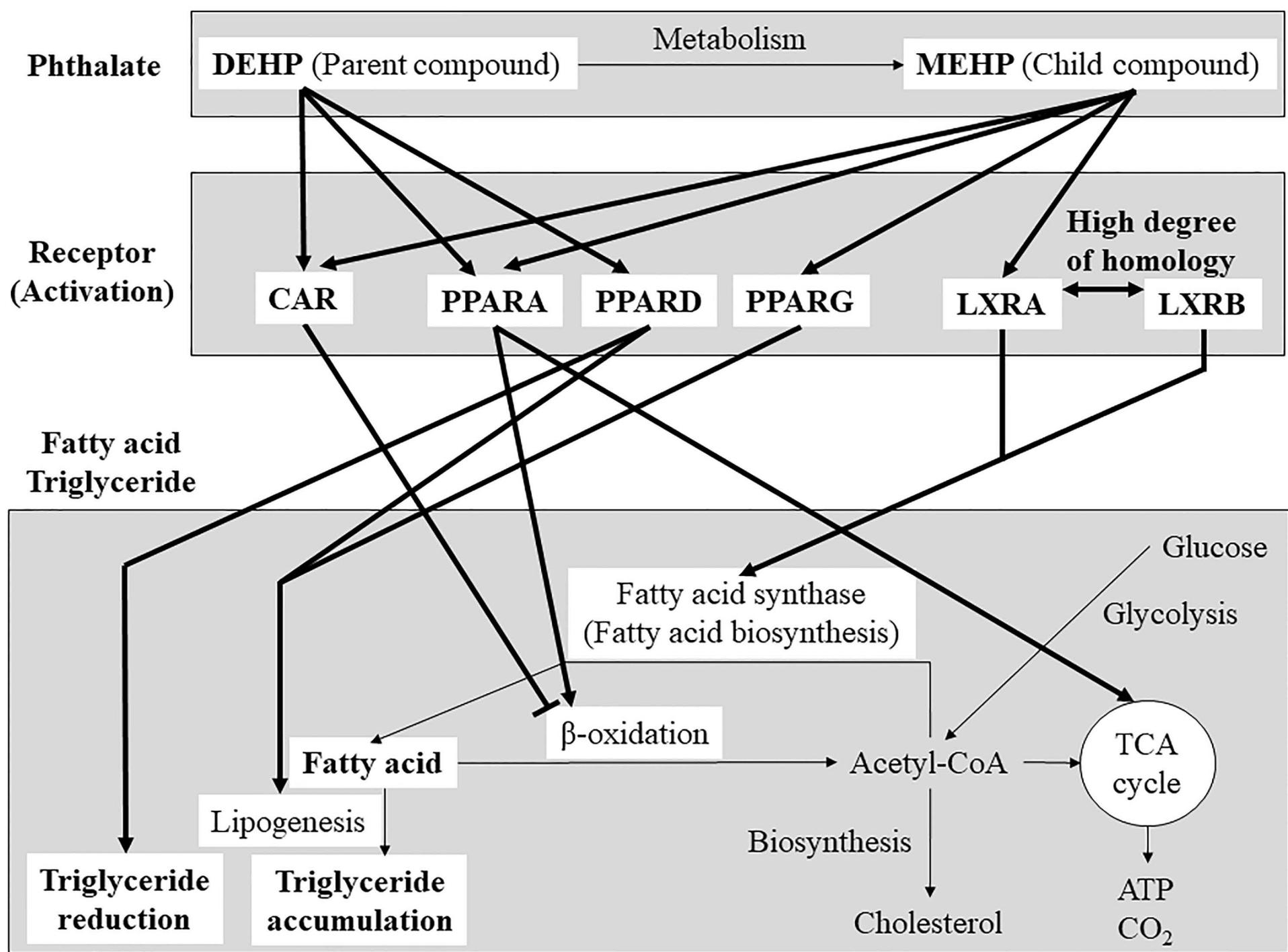
### Fig. 1. Schematic representation of the association between phthalate exposure, receptor activation, and fatty acid production

Abbreviation: ATP, adenosine triphosphate; CAR, constitutive androstane receptor; CO<sub>2</sub>, carbon dioxide; DEHP, di-(2-ethylhexyl) phthalate; LXRA, liver X receptor alpha; LXRβ, liver X receptor beta; MEHP, mono-(2-ethylhexyl) phthalate; PPARα, proliferator-activated receptor alpha; PPARδ, proliferator-activated receptor delta; PPARγ, proliferator-activated receptor (PPAR) gamma; TCA cycle, tricarboxylic acid cycle.

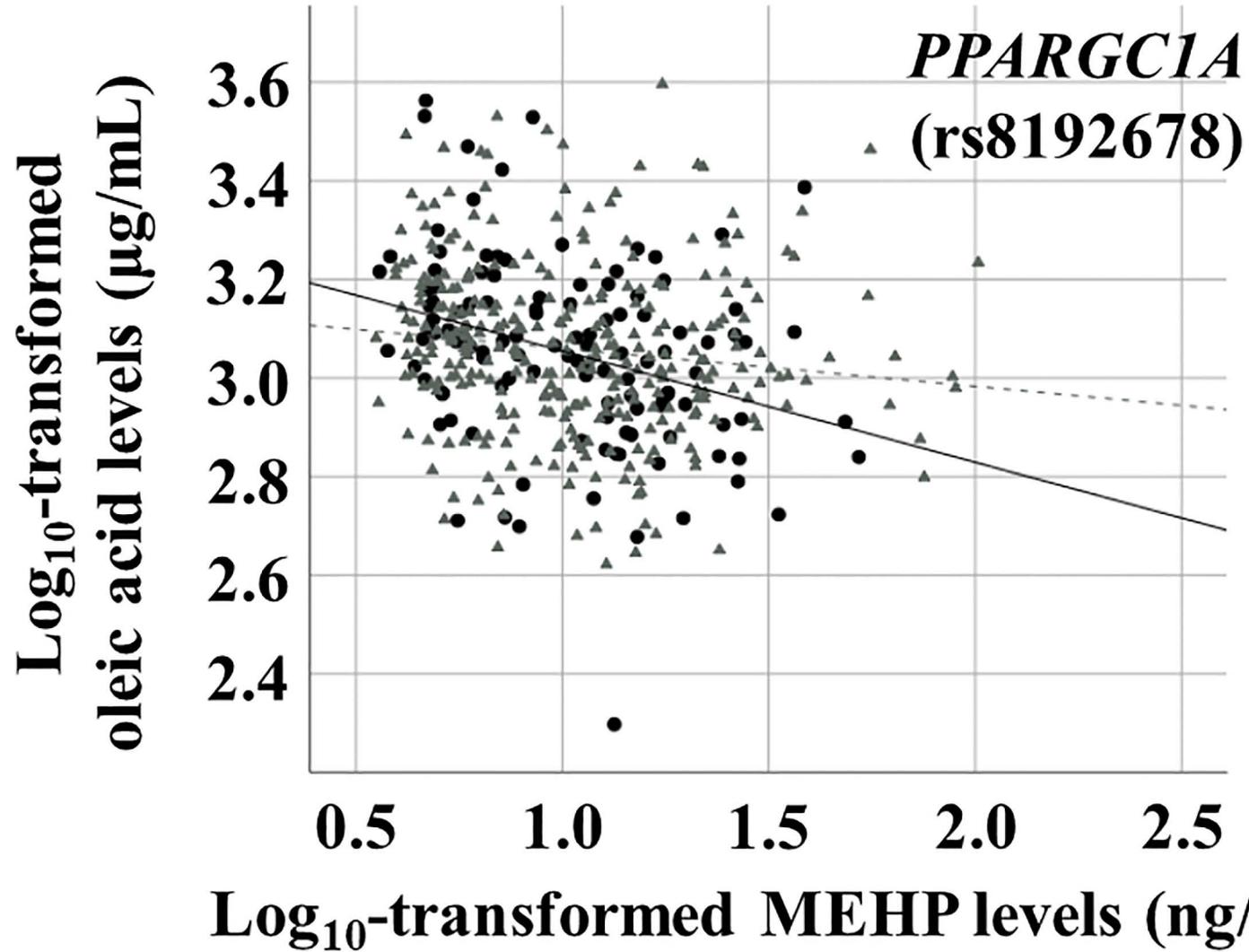
Fig. 2. Linkage disequilibrium (LD) plot for the *PPARA* (rs1800234 and rs135561), *PPARG* (rs3856806), *PPARGC1A* (rs2970847 and rs8192678), *PPARD* (rs1053049 and rs2267668), *CAR* (rs2307424 and rs2501873), *LXRA* (rs2279238), and *LXRβ* (rs1405655, rs2303044, and rs4802703) when a case-control study was conducted with the case of a lower than median oleic acid level ( $n_{case} = 218$ ) and the control of a higher than median oleic acid level ( $n_{control} = 219$ )

The value of the LD parameter (D') selected as the cut-off for linkage disequilibrium was 0.90. Red box represents an approximately perfect genetic linkage.

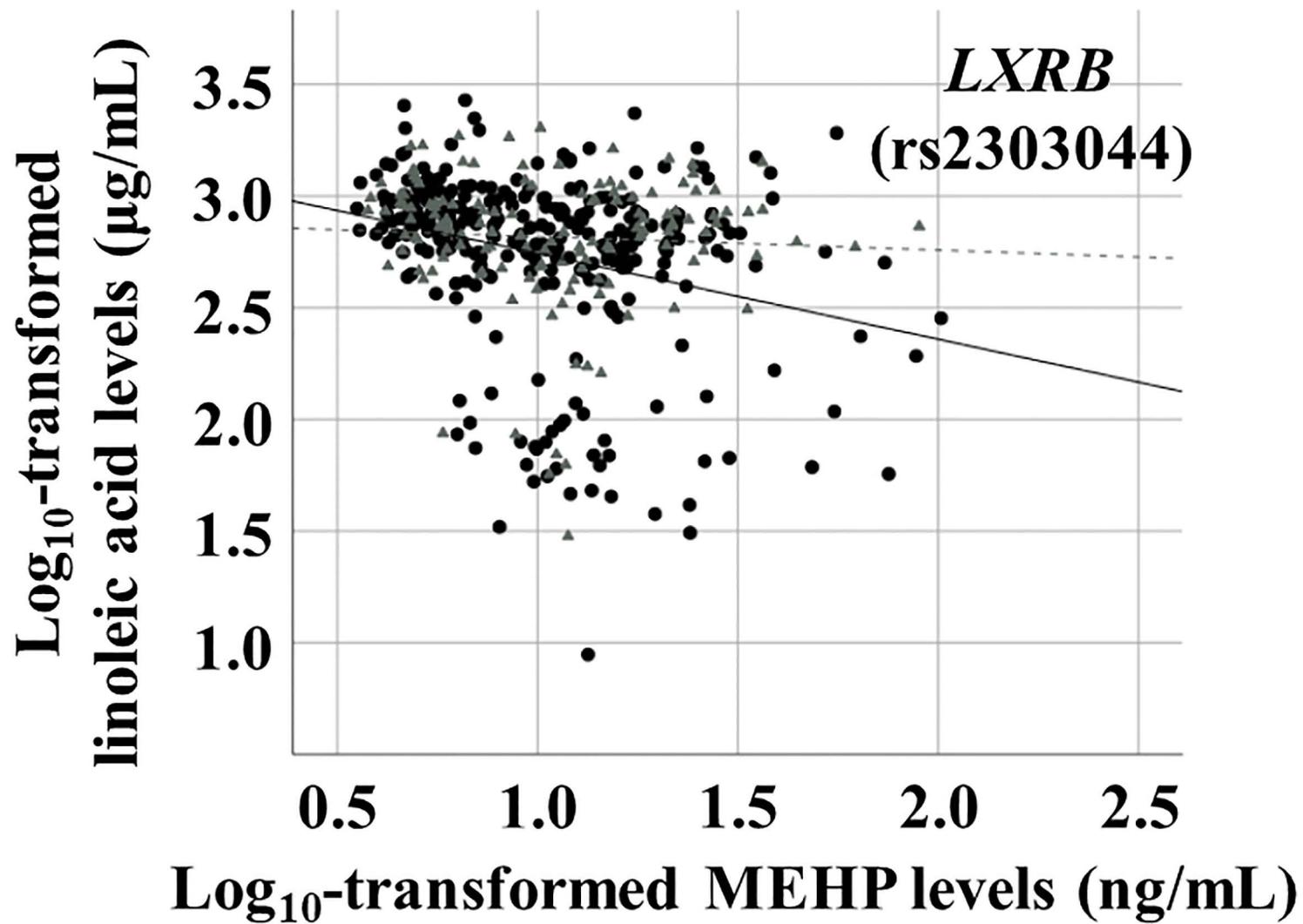
Fig. 3. Plots of the effects of gene-environment interactions (A) between *PPARGC1A* (rs8192678) and MEHP levels on oleic acid levels, (B) between *LXRβ* (rs2303044) and MEHP levels on linoleic acid levels







- GG genotype (Solid line):  $\text{Log}_{10}\text{-transformed oleic acid levels } (\mu\text{g/mL})$   
 $= 3.281 - 0.226 \times \text{Log}_{10}\text{-transformed MEHP levels (ng/mL)}$
- ▲ GA/AA genotype (Dotted line):  $\text{Log}_{10}\text{-transformed oleic acid levels } (\mu\text{g/mL})$   
 $= 3.137 - 0.077 \times \text{Log}_{10}\text{-transformed MEHP levels (ng/mL)}$



- GG genotype (Solid line):  $\text{Log}_{10}$ -transformed linoleic acid levels (μg/mL)  
 $= 3.127 - 0.384 \times \text{Log}_{10}$ -transformed MEHP levels (ng/mL)
- ▲ GA/AA genotype (Dotted line):  $\text{Log}_{10}$ -transformed linoleic acid levels (μg/mL)  
 $= 2.880 - 0.061 \times \text{Log}_{10}$ -transformed MEHP levels (ng/mL)

Table 1. Characteristics examined in the Hokkaido Study on Environment and Children's Health

(n = 437)

Characteristics	n (%), mean ± SD, or median (inter-quartile range)
Age (years) <sup>a</sup>	30.2 ± 4.8
Pre-pregnancy body mass index (BMI) (kg/m <sup>2</sup> ) <sup>a</sup>	21.3 ± 3.3
Smoking in the 3 <sup>rd</sup> trimester <sup>b</sup>	
No	357 (81.7)
Yes	80 (18.3)
Alcohol consumption during pregnancy <sup>b</sup>	
No	307 (70.3)
Yes	130 (29.7)
Parity <sup>b</sup>	
Primiparous	207 (47.4)
Multiparous	229 (52.4)
Missing data	1 (0.2)
Annual household income (million Japanese yen) <sup>b</sup>	
<5	302 (69.1)
≥5	126 (28.8)
Missing data	9 (2.1)
Blood sampling period <sup>b</sup>	
During pregnancy	281 (64.3)
After delivery	156 (35.7)
Maternal serum level <sup>c</sup>	
Mono-(2-ethylhexyl) phthalate (MEHP) (ng/mL)	10.2 (5.9, 16.3)
Perfluorooctanesulfonate (PFOS) (ng/mL)	5.2 (3.4, 7.1)
Perfluorooctanoic acid (PFOA) (ng/mL)	1.3 (0.8, 1.8)
Triglyceride (mg/100-mL)	84.5 (61.3, 117.8)
Palmitic acid (μg/mL)	1,883.2 (1,558.1, 2,445.6)
Palmitoleic acid (μg/mL)	108.5 (78.5, 160.0)
Stearic acid (μg/mL)	530.2 (433.6, 635.2)
Oleic acid (μg/mL)	1,128.8 (882.8, 1,439.4)
Linoleic acid (μg/mL)	714.2 (500.1, 946.6)
Linolenic acid (μg/mL)	10.1 (5.4, 15.3)
Arachidonic acid (μg/mL)	64.7 (44.5, 93.9)
Eicosapentaenoic acid (μg/mL)	8.5 (4.8, 14.0)
Docosahexaenoic acid (μg/mL)	25.0 (13.7, 39.0)
Maternal genotype <sup>b</sup>	
<i>PPARA</i> (T>C, dbSNP ID: rs1800234)	
TT	390 (89.2)
TC	44 (10.1)
CC	3 (0.7)
TC/CC	47 (10.8)
<i>PPARA</i> (G>A, dbSNP ID: rs135561)	
GG	388 (88.8)
GA	48 (11.0)
AA	1 (0.2)
GA/AA	49 (11.2)
<i>PPARG</i> (C>T, dbSNP ID: rs3856806)	
CC	313 (71.6)
CT	111 (25.4)
TT	13 (3.0)
CT/TT	124 (28.4)
<i>PPARGC1A</i> (C>T, dbSNP ID: rs2970847)	
CC	269 (61.6)
CT	149 (34.1)
TT	19 (4.3)
CT/TT	168 (38.4)
<i>PPARGC1A</i> (G>A, dbSNP ID: rs8192678)	
GG	120 (27.5)
GA	215 (49.2)
AA	102 (23.3)
GA/AA	317 (72.5)
<i>PPARD</i> (T>C, dbSNP ID: rs1053049)	
TT	269 (61.6)
TC	157 (35.9)
CC	11 (2.5)
TC/CC	168 (38.4)
<i>PPARD</i> (A>G, dbSNP ID: rs2267668)	
AA	287 (65.7)

AG	140 (32.0)
GG	10 (2.3)
AG/GG	150 (34.3)
<i>CAR</i> (T>C, dbSNP ID: rs2307424)	
TT	116 (26.5)
TC	235 (53.8)
CC	86 (19.7)
TC/CC	321 (73.5)
<i>CAR</i> (A>G, dbSNP ID: rs2501873)	
AA	139 (31.8)
AG	232 (53.1)
GG	66 (15.1)
AG/GG	298 (68.2)
<i>LXRA</i> (A>G, dbSNP ID: rs2279238)	
AA	184 (42.1)
AG	203 (46.5)
GG	50 (11.4)
AG/GG	253 (57.9)
<i>LXRB</i> (T>C, dbSNP ID: rs1405655)	
TT	287 (65.7)
TC	129 (29.5)
CC	21 (4.8)
TC/CC	150 (34.3)
<i>LXRB</i> (G>A, dbSNP ID: rs2303044)	
GG	297 (68.0)
GA	125 (28.6)
AA	15 (3.4)
GA/AA	140 (32.0)
<i>LXRB</i> (G>A, dbSNP ID: rs4802703)	
GG	313 (71.6)
GA	112 (25.6)
AA	12 (2.7)
GA/AA	124 (28.4)

Gene names: *CAR*, constitutive androstane receptor; *LXRA*, liver X receptor alpha; *LXRB*, liver X receptor beta; *PPARA*, peroxisome proliferator-activated receptor alpha; *PPARD*, peroxisome proliferator-activated receptor delta; *PPARG*, peroxisome proliferator-activated receptor gamma; *PPARGCIA*, peroxisome proliferator-activated receptor gamma coactivator 1-alpha.

<sup>a</sup> Mean  $\pm$  Standard deviation (SD).

<sup>b</sup> *n* (%).

<sup>c</sup> Median (inter-quartile range; IQR).

Table 2. Association between MEHP and fatty acid levels

Outcome	Exposure: MEHP		
	Crude β (95% CI)	Adjusted 1 β (95% CI)	Adjusted 2 β (95% CI)
Triglyceride	-0.110 (-0.179, -0.041)**	-0.118 (-0.186, -0.049)**	-0.063 (-0.138, 0.013)
Palmitic acid	-0.118 (-0.169, -0.067)***	-0.120 (-0.171, -0.068)***	-0.093 (-0.151, -0.035)**
Palmitoleic acid	-0.150 (-0.226, -0.074)***	-0.158 (-0.234, -0.082)***	-0.115 (-0.200, -0.031)**
Stearic acid	-0.015 (-0.063, 0.032)	-0.015 (-0.063, 0.033)	-0.038 (-0.092, 0.016)
Oleic acid	-0.113 (-0.171, -0.055)***	-0.118 (-0.176, -0.060)***	-0.089 (-0.154, -0.025)**
Linoleic acid	-0.277 (-0.397, -0.157)***	-0.277 (-0.398, -0.155)***	-0.140 (-0.272, -0.007)*
Linolenic acid	-0.288 (-0.427, -0.149)***	-0.291 (-0.431, -0.151)***	-0.163 (-0.317, -0.009)*
Arachidonic acid	-0.134 (-0.250, -0.017)*	-0.128 (-0.247, -0.010)*	-0.046 (-0.177, 0.085)
Eicosapentaenoic acid	-0.023 (-0.147, 0.102)	-0.024 (-0.150, 0.102)	-0.094 (-0.233, 0.046)
Docosahexaenoic acid	-0.011 (-0.137, 0.115)	-0.005 (-0.132, 0.123)	0.032 (-0.110, 0.175)

Abbreviations: CI, confidence interval; MEHP, mono-(2-ethylhexyl) phthalate; PFOS, perfluorooctanesulfonate.

Association between MEHP and fatty acid levels was tested in multiple linear regression models.

Crude: Non-adjusted.

Adjusted 1: Adjusted for maternal age (years, continuous), maternal smoking during the 3<sup>rd</sup> trimester (yes/no), maternal alcohol consumption during pregnancy (yes/no), annual household income (< 5/≥ 5 million Japanese yen), parity (primiparous or multiparous), and sampling period (during pregnancy or after delivery).

Adjusted 2: Adjusted for the covariates of “adjusted 1” plus log<sub>10</sub>-transformed PFOS level (ng/mL).

β (95% CI) represents the change in log<sub>10</sub>-transformed levels of triglycerides (mg/100 mL), palmitic acid (μg/mL), palmitoleic acid (μg/mL), stearic acid (μg/mL), oleic acid (μg/mL), linoleic acid (μg/mL), linolenic acid (μg/mL), arachidonic acid (μg/mL), eicosapentaenoic acid (μg/mL), or docosahexaenoic acid (μg/mL) for each 10-fold increase in MEHP levels (ng/mL).

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

Table 3. Effects of the interaction between MEHP levels and maternal *PPARGC1A* (rs8192678) genotype on fatty acid levels

Outcome	Exposure and genotype	Crude β (95% CI)	Adjusted 1 β (95% CI)	Adjusted 2 β (95% CI)
Triglyceride	MEHP	-0.193 (-0.333, -0.053)**	-0.192 (-0.332, -0.053)**	-0.132 (-0.275, 0.012)
	<i>PPARGC1A</i> -GA/AA	-0.088 (-0.256, 0.081)	-0.080 (-0.249, 0.088)	-0.072 (-0.239, 0.095)
	MEHP × <i>PPARGC1A</i> -GA/AA	0.109 (-0.052, 0.269)	0.098 (-0.063, 0.259)	0.089 (-0.071, 0.248)
		$p_{int} = 0.185$	$p_{int} = 0.233$	$p_{int} = 0.275$
Palmitic acid	MEHP	-0.206 (-0.309, -0.102)***	-0.211 (-0.317, -0.106)***	-0.182 (-0.291, -0.074)**
	<i>PPARGC1A</i> -GA/AA	-0.111 (-0.236, 0.014)	-0.117 (-0.241, 0.010)	-0.113 (-0.239, 0.014)
	MEHP × <i>PPARGC1A</i> -GA/AA	0.115 (-0.004, 0.235)	0.121 (0.000, 0.243)	0.117 (-0.004, 0.238)
		$p_{int} = 0.058$	$p_{int} = 0.050$	$p_{int} = 0.058$
Palmitoleic acid	MEHP	-0.146 (-0.301, 0.008)	-0.151 (-0.306, 0.004)	-0.103 (-0.263, 0.057)
	<i>PPARGC1A</i> -GA/AA	0.039 (-0.147, 0.226)	-0.011 (-0.190, 0.168)	0.050 (-0.136, 0.237)
	MEHP × <i>PPARGC1A</i> -GA/AA	-0.007 (-0.184, 0.171)	-0.011 (-0.190, 0.168)	-0.018 (-0.197, 0.160)
		$p_{int} = 0.940$	$p_{int} = 0.902$	$p_{int} = 0.839$
Stearic acid	MEHP	-0.082 (-0.179, 0.014)	-0.080 (-0.178, 0.018)	-0.107 (-0.208, -0.005)*
	<i>PPARGC1A</i> -GA/AA	-0.082 (-0.179, 0.014)	-0.097 (-0.215, 0.022)	-0.100 (-0.219, 0.018)
	MEHP × <i>PPARGC1A</i> -GA/AA	0.089 (-0.022, 0.200)	0.087 (-0.027, 0.200)	0.091 (-0.022, 0.204)
		$p_{int} = 0.116$	$p_{int} = 0.134$	$p_{int} = 0.116$
Oleic acid	MEHP	-0.226 (-0.343, -0.109)***	-0.232 (-0.350, -0.114)***	-0.200 (-0.322, -0.079)**
	<i>PPARGC1A</i> -GA/AA	-0.144 (-0.285, -0.003)*	-0.146 (-0.288, -0.004)*	-0.141 (-0.283, 0.000)*
	MEHP × <i>PPARGC1A</i> -GA/AA	0.149 (0.014, 0.283)	0.150 (0.014, 0.286)	0.145 (0.010, 0.281)
		$p_{int} = 0.030$	$p_{int} = 0.031$	$p_{int} = 0.036$
Linoleic acid	MEHP	-0.377 (-0.620, -0.133)**	-0.384 (-0.630, -0.137)**	-0.230 (-0.480, 0.020)
	<i>PPARGC1A</i> -GA/AA	-0.095 (-0.389, 0.199)	-0.105 (-0.403, 0.193)	-0.084 (-0.375, 0.207)
	MEHP × <i>PPARGC1A</i> -GA/AA	0.130 (-0.150, 0.409)	0.139 (-0.145, 0.424)	0.116 (-0.162, 0.395)
		$p_{int} = 0.363$	$p_{int} = 0.337$	$p_{int} = 0.412$
Linolenic acid	MEHP	-0.383 (-0.666, -0.100)**	-0.369 (-0.655, -0.084)*	-0.227 (-0.518, 0.064)
	<i>PPARGC1A</i> -GA/AA	-0.067 (-0.409, 0.274)	-0.049 (-0.393, 0.295)	-0.031 (-0.370, 0.308)
	MEHP × <i>PPARGC1A</i> -GA/AA	0.123 (-0.202, 0.448)	0.100 (-0.229, 0.429)	0.080 (-0.244, 0.405)
		$p_{int} = 0.459$	$p_{int} = 0.551$	$p_{int} = 0.627$
Arachidonic acid	MEHP	-0.085 (-0.322, 0.153)	-0.086 (-0.327, 0.156)	0.008 (-0.240, 0.256)
	<i>PPARGC1A</i> -GA/AA	0.108 (-0.178, 0.394)	0.100 (-0.191, 0.391)	0.112 (-0.176, 0.401)
	MEHP × <i>PPARGC1A</i> -GA/AA	-0.067 (-0.340, 0.205)	-0.059 (-0.337, 0.219)	-0.073 (-0.349, 0.203)
		$p_{int} = 0.629$	$p_{int} = 0.676$	$p_{int} = 0.603$
Eicosapentaenoic acid	MEHP	-0.050 (-0.305, 0.204)	-0.037 (-0.296, 0.221)	-0.115 (-0.381, 0.152)
	<i>PPARGC1A</i> -GA/AA	0.040 (-0.266, 0.346)	0.055 (-0.256, 0.365)	0.045 (-0.264, 0.355)
	MEHP × <i>PPARGC1A</i> -GA/AA	0.033 (-0.259, 0.324)	0.014 (-0.284, 0.311)	0.024 (-0.273, 0.320)
		$p_{int} = 0.826$	$p_{int} = 0.928$	$p_{int} = 0.876$
Docosahexaenoic acid	MEHP	0.072 (-0.183, 0.327)	0.075 (-0.185, 0.335)	0.117 (-0.153, 0.386)
	<i>PPARGC1A</i> -GA/AA	0.186 (-0.122, 0.493)	0.181 (-0.132, 0.495)	0.187 (-0.127, 0.500)
	MEHP × <i>PPARGC1A</i> -GA/AA	-0.114 (-0.407, 0.180)	-0.110 (-0.410, 0.190)	-0.116 (-0.416, 0.184)
		$p_{int} = 0.447$	$p_{int} = 0.472$	$p_{int} = 0.447$

Abbreviations: CI, confidence interval; MEHP, mono-(2-ethylhexyl) phthalate; PFOS, perfluorooctanesulfonate; *PPARGC1A*, peroxisome proliferator-activated receptor gamma co-activator 1-alpha. Association between MEHP and any fatty levels was tested in multiple linear regression models.

Crude: Non-adjusted.

Adjusted 1: Adjusted for maternal age (years, continuous), maternal smoking during the 3<sup>rd</sup> trimester (yes/no), maternal alcohol consumption during pregnancy (yes/no), annual household income (< 5/≥ 5 million Japanese yen), parity (primiparous or multiparous), and sampling period (during pregnancy or after delivery).

Adjusted 2: Adjusted for the covariates of “adjusted 1” plus log<sub>10</sub>-transformed PFOS level (ng/mL).

β (95% CI) represents the change in log<sub>10</sub>-transformed levels of triglycerides (mg/100 mL), palmitic acid (μg/mL), palmitoleic acid (μg/mL), stearic acid (μg/mL), oleic acid (μg/mL), linoleic acid (μg/mL), linolenic acid (μg/mL), arachidonic acid (μg/mL), eicosapentaenoic acid (μg/mL), or docosahexaenoic acid (μg/mL).

$p_{int}$  represents the  $p$ -value for the interaction.

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

Table 4. Effects of the interaction between MEHP levels and maternal *LXRB* (rs2303044) genotype on fatty acid levels

Outcome	Exposure and genotype	Crude β (95% CI)	Adjusted 1 β (95% CI)	Adjusted 2 β (95% CI)
Triglyceride	MEHP	-0.136 (-0.220, -0.053)**	-0.152 (-0.235, -0.069)***	-0.095 (-0.183, -0.006)*
	<i>LXRB</i> -GA/AA	-0.044 (-0.202, 0.114)	-0.075 (-0.232, 0.082)	-0.067 (-0.223, 0.088)
	MEHP × <i>LXRB</i> -GA/AA	0.077 (-0.071, 0.225)	0.102 (-0.045, 0.249)	0.098 (-0.047, 0.244)
		$p_{int} = 0.307$	$p_{int} = 0.175$	$p_{int} = 0.184$
Palmitic acid	MEHP	-0.142 (-0.204, -0.080)***	-0.145 (-0.208, -0.082)***	-0.118 (-0.186, -0.050)**
	<i>LXRB</i> -GA/AA	-0.069 (-0.186, 0.048)	-0.075 (-0.194, 0.043)	-0.072 (-0.190, 0.047)
	MEHP × <i>LXRB</i> -GA/AA	0.074 (-0.035, 0.184)	0.079 (-0.032, 0.190)	0.077 (-0.034, 0.188)
		$p_{int} = 0.183$	$p_{int} = 0.165$	$p_{int} = 0.172$
Palmitoleic acid	MEHP	-0.173 (-0.265, -0.080)***	-0.186 (-0.279, -0.094)***	-0.143 (-0.242, -0.043)**
	<i>LXRB</i> -GA/AA	-0.053 (-0.227, 0.122)	-0.079 (-0.254, 0.095)	-0.074 (-0.247, 0.100)
	MEHP × <i>LXRB</i> -GA/AA	0.068 (-0.096, 0.231)	0.098 (-0.076, 0.251)	0.085 (-0.078, 0.248)
		$p_{int} = 0.416$	$p_{int} = 0.292$	$p_{int} = 0.304$
Stearic acid	MEHP	-0.014 (-0.072, 0.043)	-0.015 (-0.074, 0.044)	-0.038 (-0.101, 0.026)
	<i>LXRB</i> -GA/AA	0.013 (-0.097, 0.122)	0.010 (-0.100, 0.121)	0.007 (-0.103, 0.118)
	MEHP × <i>LXRB</i> -GA/AA	-0.005 (-0.107, 0.098)	-0.002 (-0.106, 0.102)	-0.001 (-0.105, 0.103)
		$p_{int} = 0.930$	$p_{int} = 0.968$	$p_{int} = 0.987$
Oleic acid	MEHP	-0.132 (-0.202, -0.062)***	-0.142 (-0.212, -0.071)***	-0.112 (-0.188, -0.036)**
	<i>LXRB</i> -GA/AA	-0.053 (-0.185, 0.080)	-0.071 (-0.204, 0.062)	-0.067 (-0.200, 0.066)
	MEHP × <i>LXRB</i> -GA/AA	0.059 (-0.066, 0.183)	0.073 (-0.052, 0.198)	0.071 (-0.053, 0.196)
		$p_{int} = 0.355$	$p_{int} = 0.251$	$p_{int} = 0.260$
Linoleic acid	MEHP	-0.384 (-0.527, -0.240)***	-0.389 (-0.534, -0.243)***	-0.246 (-0.400, -0.092)**
	<i>LXRB</i> -GA/AA	-0.248 (-0.519, 0.024)	-0.268 (-0.543, 0.006)	-0.249 (-0.517, 0.019)
	MEHP × <i>LXRB</i> -GA/AA	0.323 (0.068, 0.577)	0.338 (0.081, 0.595)	0.330 (0.079, 0.581)
		$p_{int} = 0.013$	$p_{int} = 0.010$	$p_{int} = 0.010$
Linolenic acid	MEHP	-0.355 (-0.523, -0.187)***	-0.371 (-0.540, -0.202)***	-0.237 (-0.417, -0.057)*
	<i>LXRB</i> -GA/AA	-0.114 (-0.432, 0.203)	-0.162 (-0.481, 0.156)	-0.144 (-0.458, 0.170)
	MEHP × <i>LXRB</i> -GA/AA	0.197 (-0.100, 0.495)	0.238 (-0.061, 0.536)	0.230 (-0.064, 0.525)
		$p_{int} = 0.193$	$p_{int} = 0.119$	$p_{int} = 0.124$
Arachidonic acid	MEHP	-0.219 (-0.359, -0.079)**	-0.215 (-0.358, -0.073)**	-0.128 (-0.280, 0.025)
	<i>LXRB</i> -GA/AA	-0.182 (-0.448, 0.083)	-0.188 (-0.457, 0.082)	-0.176 (-0.442, 0.091)
	MEHP × <i>LXRB</i> -GA/AA	0.257 (0.008, 0.505)	0.259 (0.007, 0.511)	0.254 (0.004, 0.504)
		$p_{int} = 0.043$	$p_{int} = 0.046$	$p_{int} = 0.046$
Eicosapentaenoic acid	MEHP	0.010 (-0.141, 0.161)	0.003 (-0.150, 0.156)	-0.069 (-0.234, 0.096)
	<i>LXRB</i> -GA/AA	0.075 (-0.210, 0.361)	0.052 (-0.237, 0.341)	0.042 (-0.246, 0.330)
	MEHP × <i>LXRB</i> -GA/AA	-0.097 (-0.365, 0.170)	-0.078 (-0.348, 0.193)	-0.073 (-0.343, 0.196)
		$p_{int} = 0.474$	$p_{int} = 0.572$	$p_{int} = 0.592$
Docosahexaenoic acid	MEHP	-0.088 (-0.238, 0.063)	-0.082 (-0.235, 0.072)	-0.039 (-0.205, 0.127)
	<i>PPARGC1A</i> -GA/AA	-0.114 (-0.400, 0.171)	-0.114 (-0.404, 0.176)	-0.108 (-0.398, 0.181)
	MEHP × <i>PPARGC1A</i> -GA/AA	0.223 (-0.045, 0.490)	0.223 (-0.049, 0.494)	0.220 (-0.051, 0.491)
		$p_{int} = 0.103$	$p_{int} = 0.108$	$p_{int} = 0.111$

Abbreviations: CI, confidence interval; MEHP, mono-(2-ethylhexyl) phthalate; PFOS, perfluorooctanesulfonate; *LXRB*, liver X receptor beta. Association between MEHP and fatty acid levels was tested in multiple linear regression models.

Crude: Non-adjusted.

Adjusted 1: Adjusted for maternal age (years, continuous), maternal smoking during the 3<sup>rd</sup> trimester (yes/no), maternal alcohol consumption during pregnancy (yes/no), annual household income (< 5/≥ 5 million Japanese yen), parity (primiparous or multiparous), and sampling period (during pregnancy or after delivery).

Adjusted 2: Adjusted for the covariates of “adjusted 1” plus log<sub>10</sub>-transformed PFOS level (ng/mL).

β (95% CI) represents the change in log<sub>10</sub>-transformed levels of triglycerides (mg/100 mL), palmitic acid (μg/mL), palmitoleic acid (μg/mL), stearic acid (μg/mL), oleic acid (μg/mL), linoleic acid (μg/mL), linolenic acid (μg/mL), arachidonic acid (μg/mL), eicosapentaenoic acid (μg/mL), or docosahexaenoic acid (μg/mL).

$p_{int}$  represents the  $p$ -value for the interaction.

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .