Associations between maternal mono-(2-ethylhexyl) phthalate levels, nuclear receptor gene polymorphisms, and fatty acid levels in pregnant Japanese women in the Hokkaido study

Sumitaka Kobayashi[a], Fumihiro Sata[a,b], Chihiro Miyashita[a], Atsuko Ikeda-Araki[a,c], Houman Goudarzi[a,d], Tamie Nakajima[e], Reiko Kishi[a,*]

a. Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Japan

b. Health Center, Chuo University, Tokyo, Japan

c. Faculty of Health Sciences, Hokkaido University, Sapporo, Japan

d. Faculty of Medicine and Graduate School of Medicine, Center for Medical Education and International Relations, Hokkaido University, Sapporo, Japan

e. College of Life and Health Sciences, Chubu University, Kasugai, Japan

***Corresponding author**

Reiko Kishi, MD, PhD, MPH

Center for Environmental and Health Sciences, Hokkaido University, North-12, West-7, Kita-ku, Sapporo 060-0812, Japan

E-mail: rkishi@med.hokudai.ac.jp

Tel: +81-(0)11-706-4746

Fax: +81-(0)11-706-4725

Highlights

- Mono-(2-ethylhexyl) phthalate was associated with fatty acids.
- Genotype *PPARGC1A* (rs8192678) affected oleic acid level.
- Genotype *LXRB* (rs2203044) affected linoleic acid level.
- Gene-environment interaction persisted after adjusting for perfluorooctanesulfonate.

Abstract

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

influence of log_{10}-transformed MEHP levels and maternal genotypes on log_{10}-transformed fatty acid levels. When the effects of the interaction between MEHP levels and the maternal *PPARGC1A* (rs8192678) genotype on oleic acid levels were evaluated, the estimated changes (95% confidence intervals) in oleic acid levels against MEHP levels, maternal *PPARGC1A* (rs8192678)-GA/AA genotype, and the interaction between them showed a mean reduction of 0.200 (0.079, 0.322), mean reduction of 0.141 (0.000, 0.283), and mean increase of 0.145 (0.010, 0.281), respectively, after adjusting for the perfluorooctanesulfonate level. The effects of the interaction between MEHP levels and maternal *LXRB* (rs2303044) genotype on linoleic acid levels was also significant (p_{int} = 0.010). In conclusion, the interaction between MEHP and the maternal genotypes *PPARGC1A* (rs8192678) and *LXRB* (rs2303044) decreased fatty acid levels. Further, the interaction between MEHP and *PPARGC1A* (rs8192678) may have a greater effect on fatty acid levels than the interaction between PFOS and *PPARGC1A*.

Keywords

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

Abbreviations

ABC, ATP-binding cassette

ABCB1, ATP-binding cassette, sub-family B, member 1

ABCC2, ATP-binding cassette, sub-family C, member 2

BMI, body mass index

CAR, constitutive androstane receptor

CI, confidence interval

DEHP, di-(2-ethylhexyl) phthalate

ESR1, estrogen receptor 1

GC/MS, gas chromatography-mass spectrometry

HWE, Hardy-Weinberg equilibrium

LC/MS/MS, liquid chromatography-tandem mass spectrometry

LD, linkage disequilibrium

LOD, limit of detection

LXR, liver X receptor

LXRA, liver X receptor alpha

LXRB, liver X receptor beta

MDR1, multiple drug resistance 1

MEHP, mono-(2-ethylhexyl) phthalate

MRP2, multidrug resistance-associated protein 2

NCBI, National Center for Biological Information

PCR, polymerase chain reaction

PFAS, perfluoroalkyl substance

PON2, paraoxonase-2

PFOA, perfluorooctanoic acid

PFOS, perfluorooctanesulfonate

PPAR, peroxisome proliferator-activated receptor

PPARA, peroxisome proliferator-activated receptor alpha

PPARD, peroxisome proliferator-activated receptor delta

PPARG, peroxisome proliferator-activated receptor gamma

PPARGC1A, peroxisome proliferator-activated receptor gamma coactivator 1A

PVC, polyvinyl chloride

SD, standard deviation

SNP, single nucleotide polymorphism

SREBP1c, sterol response element-binding protein 1c

TCA cycle, tricarboxylic acid cycle

2D:4D, ratio of the lengths of the second and fourth digits

1. Introduction

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

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

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

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

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

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

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

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

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

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

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

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

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 [41]. 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 from the study. Of the remaining subjects, 437 participants provided complete data on the levels of MEHP, PFOS, PFOA, fatty acid, and maternal genotypes.

2.2. Ethical approval

Written informed consent was obtained from all participants. All procedures were conducted in accordance with the principles of the Declaration of Helsinki. 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 age, height before pregnancy, weight before pregnancy, annual household income, smoking in the 3rd trimester, and alcohol consumption during pregnancy. Maternal records were also obtained to collect parity information.

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

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

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

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

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

447 blood samples, 228 were collected during pregnancy and 159 were obtained after delivery owing to anemia during pregnancy. All samples exceeded 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).

2.6. Assessment of maternal genotypes

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

PPARD (T>C, dbSNP ID: rs1053049; A>G, dbSNP ID: rs2267668), *PPARG* (C>T, His449His; dbSNP ID: rs3856806), *PPARGC1A* (C>T, Thr394Thr; dbSNP ID: rs2970847; G>A, dbSNP ID: rs8192678), *CAR* (T>C, Pro180Pro; dbSNP ID: rs2307424; A>G, dbSNP ID: rs2501873), *LXRA* (C>T, Ser99Ser; dbSNP ID: rs2279238), and *LXRB* (T>C, dbSNP ID: rs1405655; G>A, dbSNP ID: rs2303044; G>A; dbSNP ID: rs4802703) [45-55]. The evaluation was performed based on the analysis of high-throughput gene expression of preamplification, real-time polymerase chain reaction (PCR) performed with dynamic chips, and TaqMan gene expression measurements. The assessment protocol has been described previously [36]. Approximately 5% or more minor alleles among pregnant Japanese women are necessary to secure statistical powers for examining the adverse health outcome. All 13 genetic polymorphisms satisfied a minor allele frequency of > 5%.

2.7. Statistical analyses

Of the 437 participants, data of one (0.2%) and nine (2.1%) samples corresponding to parity and annual household income, respectively were missing. Using simple imputation, the participants were assigned to the parity group of multiparous and annual household income of < 5 million Japanese yen (the most frequent group). First, we examined the characteristics of the participants. 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 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), and linkage disequilibrium (LD) was evaluated using linkage analyses. Fourth, MEHP, PFOS, PFOA, and fatty acid 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 MEHP and fatty acid levels in both crude and adjusted models. Maternal age, maternal smoking in the 3rd trimester, maternal alcohol consumption during pregnancy, parity, annual household income, and maternal blood sampling periods (up to this covariate, their covariates applied to both adjusted 1 and 2), and PFOS levels (adjusted only 2) were adjusted in the multiple linear regression models, except in the crude models. Fifth, multiple linear regression analyses were used to evaluate the interaction between MEHP and PFOS or PFOA levels with fatty acid levels in both the crude and adjusted (adjusted only 1) models. The covariates were the same as those used in the fourth analysis. Sixth, multiple linear regression analyses were used to evaluate the interaction between MEHP levels and maternal genotypes with fatty acid levels in both the crude and adjusted (both adjusted 1 and 2) models. The covariates were the same as those used in the fourth analysis. Seventh, multiple linear regression analyses were used to evaluate the association between MEHP and fatty acid levels in both the crude and adjusted (both adjusted 1 and 2) models after stratification based on maternal genotypes *PPARGC1A* (rs8192678) and *LXRB* (rs2303044), because only the effects of the

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

Data were considered statistically significant at $p < 0.05$. 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) [56].

3. Results

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

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, 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 (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, and 25.0 (13.7, 39.0) μg/mL, respectively. The distribution of 12 SNPs satisfied HWE (chi-squared test: all $p > 0.05$) in 437 participants, except for *PPARD* (rs1053049; chi-squared value = 4.636; $p = 0.031$). Moreover, no associations were observed between MEHP levels and maternal genotypes and between fatty acid levels and maternal genotypes (data not shown).

The LD plot of the 13 SNPs is shown in Fig. 2. The LD parameter values (D') for *PPARGC1A* (rs2970847 and rs8192678), *PPARD* (rs1053049 and rs2267668), and *LXRB* (rs1405655, rs2303044, and rs4802703) were ≥ 0.90. Except for these values, D' was less than 0.90.

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

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

The combined effects of the interactions of MEHP levels and maternal *PPARGC1A* (rs8192678) genotype on fatty acid levels are presented in Table 3 (see also Fig. 3.A.). Multiple linear regression analysis showed a significant interaction between MEHP levels and maternal *PPARGC1A* (rs8192678) genotype on oleic acid levels after adjusting for covariates, including PFOS levels. When the effect of the interaction between MEHP levels and maternal *PPARGC1A* (rs8192678) genotype on oleic acid levels was analyzed, the estimated changes (95% CI) in oleic acid levels against an increase of one unit in MEHP levels, those against maternal *PPARGC1A* (rs8192678)-GA/AA genotype, and those against interaction between MEHP levels and maternal *PPARGC1A* (rs8192678)-GA/AA genotype showed a mean reduction of 0.200 (0.079, 0.322), a mean reduction of 0.141 (0.000, 0.283), and a mean increase of 0.145 (0.010, 0.281) (p_{int} = 0.036), respectively, after adjusting for covariates, including PFOS levels. The effects of MEHP levels on oleic acid levels stratified by maternal *PPARGC1A* (rs8192678) genotype were analyzed. Estimated changes (95% CI) in oleic acid levels against an increase of one unit in MEHP levels involved a mean reduction of 0.130 (-0.271, 0.010) in mothers with the GG genotype and a mean

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

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

(rs3856806), *PPARGC1A* (rs2970847), *PPARD* (rs1053049 and rs2267668), *CAR* (rs2307424 and rs2501873), *LXRA* (rs2279238), and *LXRB* (rs1405655 and rs4802703) with fatty acid levels (data not shown).

4. Discussion

We examined the association between MEHP levels and fatty acid levels while considering the impact of PFOS levels in statistical analyses, since we found an association between the PFOS and fatty acid levels during pregnancy in our previous study [36], and the MEHP levels were correlated with the PFOS levels in this study (Spearman's $\rho = 0.467$; $p < 0.001$). Our results showed that the interactions between MEHP and the maternal *PPARGC1A* (rs8192678) genotypes, and that between MEHP and the maternal *LXRB* (rs2303044) genotype during pregnancy affected on the oleic acid and linoleic acid levels in maternal blood, respectively. The results of this study suggest that the prenatal gene-environment interactions MEHP and specific maternal genotypes modify the fatty acid levels in maternal blood. In a cross-sectional study across 184 countries, a decrease in the total long-chain omega-3 polyunsaturated fatty acid level in pregnant individuals (mean: 310 mg/day) was shown to be associated with an increased preterm birth rate [57]. In another study (mean linoleic acid: 19.07 ng/mL), infants of pregnant individuals with linoleic acid levels of 16.95-18.37 ng/mL in maternal plasma were increase of 11.0-g compared to pregnant

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

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

Although MEHP levels do not directly affect the PFAS levels in pregnant Japanese women, the interaction between MEHP and the maternal *PPARGC1A* (rs8192678) genotype affected oleic acid levels after adjusting for PFOS levels. In our previous study, the interaction between PFOS and the maternal *PPARGC1A* (rs8192678) genotype affected triglyceride, palmitic acid, palmitoleic acid, and oleic acid levels [36]. No effects of the interaction between MEHP and PFOS levels on triglyceride, palmitic acid, palmitoleic acid, and oleic acid levels, and correlation between MEHP and PFOS levels (Spearman's $\rho = 0.467$; $p < 0.001$), correlation between oleic acid and triglyceride (Spearman's $\rho = 0.628$; $p < 0.001$), palmitic acid (Spearman's $\rho = 0.912$; $p < 0.001$), and palmitoleic acid (Spearman's $\rho = 0.751$; $p < 0.001$) levels were observed in this study. This suggests that the interaction between MEHP and *PPARGC1A* (rs8192678) has a greater effect on fatty acid levels than the interaction between PFOS and *PPARGC1A* (rs8192678). Furthermore, it has been suggested that activation of PPAR by MEHP may increase xenobiotic metabolism [60].

In this study, two *PPARGC1A* genes (rs2970847 and rs8192678) showed LD. The *PPARGC1A* (rs8192678) GG genotype is associated with an increased risk of metabolic syndrome [61] and unstable angina [62], and individuals with this genotype show lower reductions in total cholesterol after consumption of a low-fat diet [63] than those with the GA/AA genotype. Therefore, we speculated that the rate of lipid metabolism of individuals with the *PPARGC1A*

(rs8192678) GG genotype was lower than that of individuals with the GA/AA genotype. We found that a decrease in the rate of lipid metabolism was associated with the effect of the interaction between MEHP and the maternal *PPARGC1A* (rs8192678) genotype on oleic acid levels. Oleic acid is secreted by astrocytes and used by neurons for the synthesis of phospholipids. Further, it is specifically incorporated into growth cones to promote axonal growth [64]. Hence, it may be that reduction in oleic acid levels affects the long-term suppression of brain differentiation.

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

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

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

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

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

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

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

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

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

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

5. Conclusion

Our study demonstrated that the maternal genotype *PPARGC1A* (rs8192678) modified the association between MEHP and oleic acid levels, and maternal genotype *LXRB* (rs2303044) modified the association between MEHP and linoleic acid levels. Further, the interaction between MEHP and *PPARGC1A* (rs8192678) likely has a greater effect on fatty acid levels than the

interaction between PFOS and *PPARGC1A* (rs8192678). The results of this study suggest that certain genotypes may be associated with a preventive function involved in remaining adequate fatty acid levels by maintaining low DEHP levels.

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Conflicts of interest

The authors declare no conflicts of interest.

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Figure legends

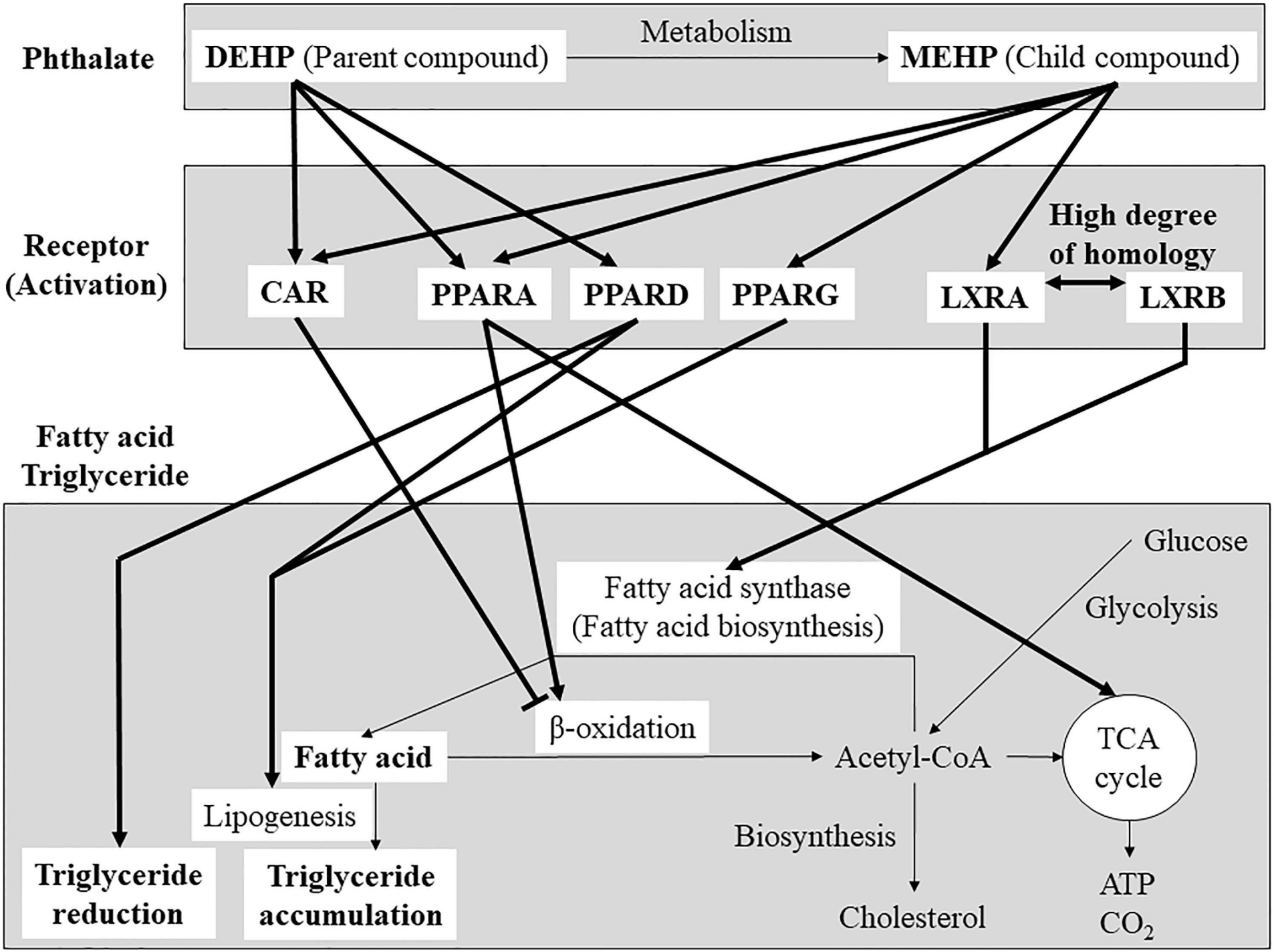
Fig. 1. Schematic representation of the association between phthalate exposure, receptor activation, and fatty acid production

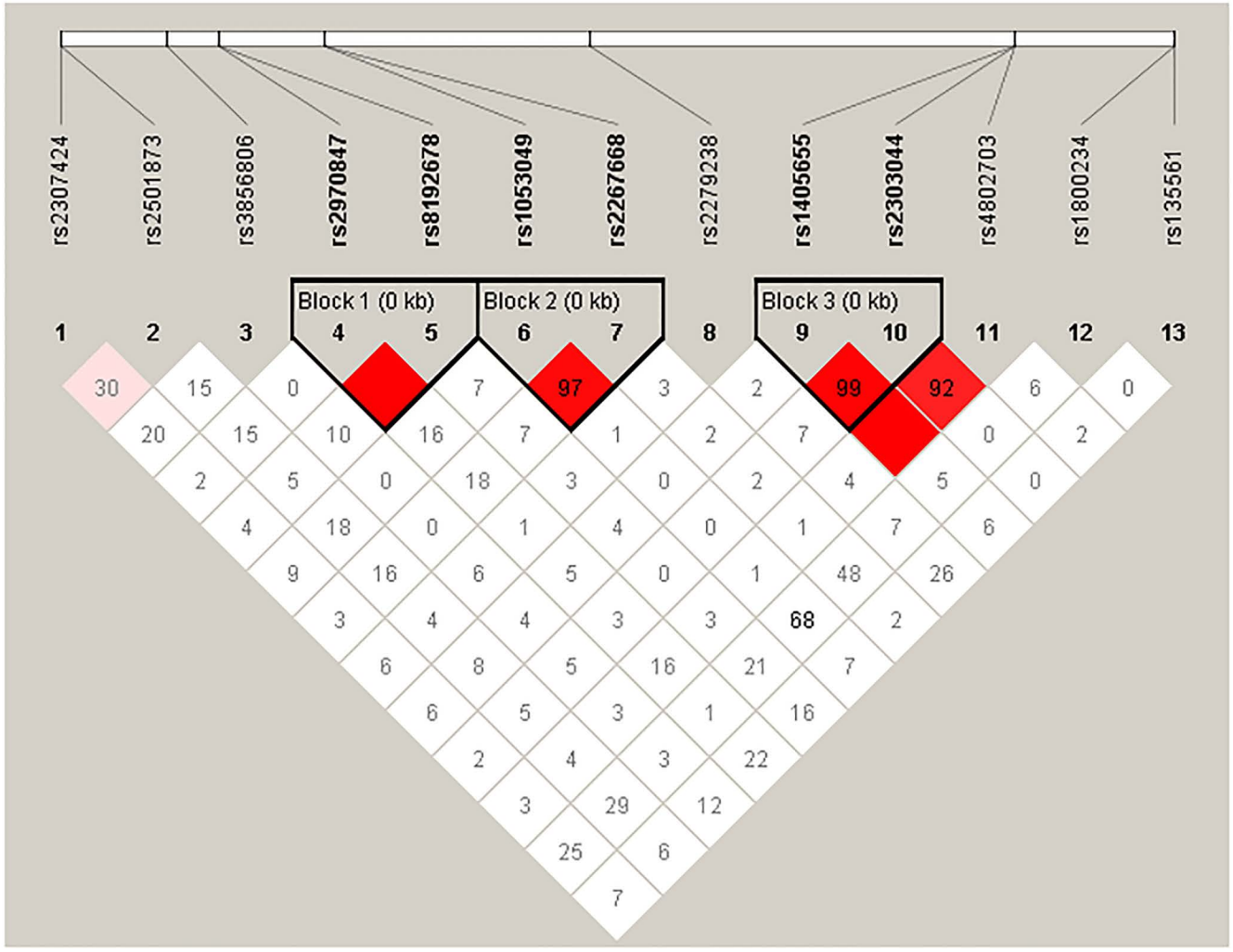
Abbreviation: ATP, adenosine triphosphate; CAR, constitutive androstane receptor; CO_2, carbon dioxide; DEHP, di-(2-ethylhexyl) phthalate; LXRA, liver X receptor alpha; LXRB, liver X receptor beta; MEHP, mono-(2-ethylhexyl) phthalate; PPARA, proliferator-activated receptor alpha; PPARD, proliferator-activated receptor delta; PPARG, 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 *LXRB* (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 *LXRB* (rs2303044) and MEHP levels on linoleic acid levels


Phthalate
DEHP (Parent compound)
Metabolism
MEHP (Child compound)
Receptor (Activation)
CAR
PPARA
PPARD
PPARG
LXRA
LXRB
High degree of homology
Fatty acid
Triglyceride
Glucose
Glycolysis
Fatty acid synthase (Fatty acid biosynthesis)
β-oxidation
Fatty acid
Acetyl-CoA
TCA cycle
Lipogenesis
Biosynthesis
Triglyceride reduction
Triglyceride accumulation
Cholesterol
ATP
CO_2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rs1800234
rs135561
Block 1 (0 kb)
Block 2 (0 kb)
Block 3 (0 kb)
1 2 3 4 5 6 7 8 9 10 11 12 13
30 15 0 7 97 3 2 99 92 6 0
20 15 10 16 7 1 2 7 0 2
2 5 0 18 3 0 2 4 5 0
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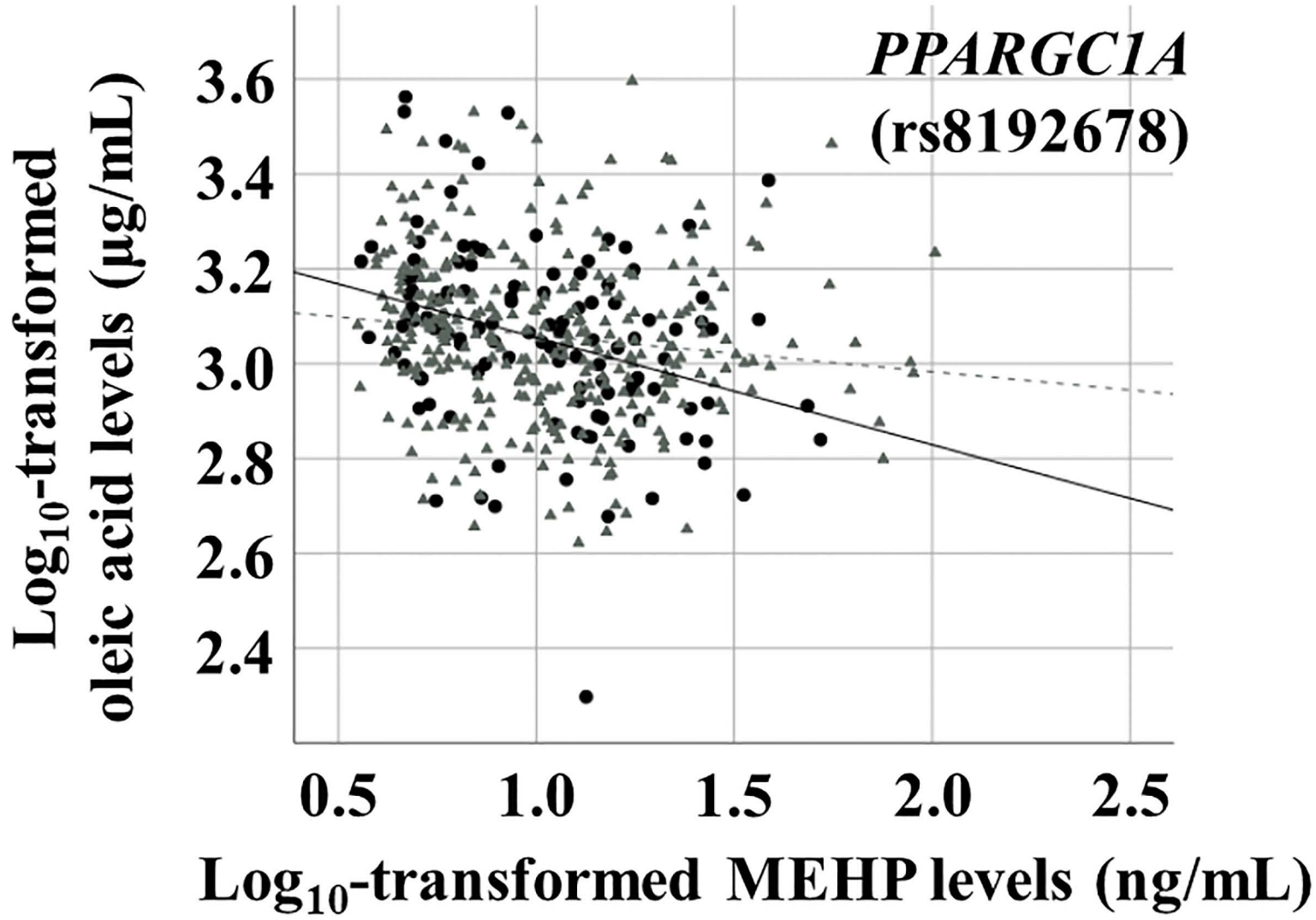
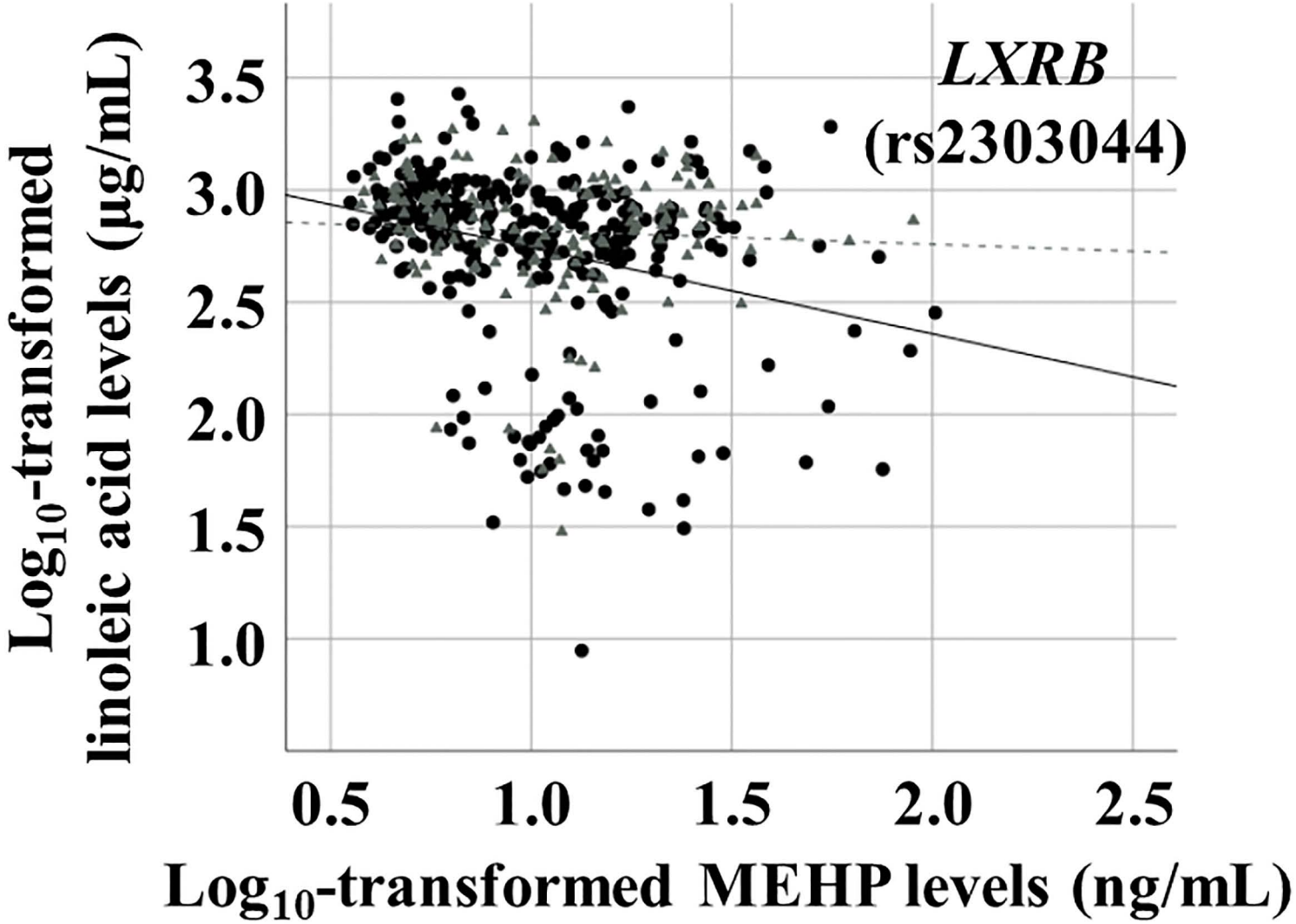
25 6
7



● GG genotype (Solid line): Log_{10}-transformed oleic acid levels (μg/mL)
$= 3.281 - 0.226 \times \text{Log}_{10}$-transformed MEHP levels (ng/mL)

▲ GA/AA genotype (Dotted line): Log_{10}-transformed oleic acid levels (μg/mL)
$= 3.137 - 0.077 \times \text{Log}_{10}$-transformed MEHP levels (ng/mL)



● GG genotype (Solid line): 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): 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)[a]	30.2 ± 4.8
Pre-pregnancy body mass index (BMI) (kg/m^2)[a]	21.3 ± 3.3
Smoking in the 3rd trimester[b]	
No	357 (81.7)
Yes	80 (18.3)
Alcohol consumption during pregnancy[b]	
No	307 (70.3)
Yes	130 (29.7)
Parity[b]	
Primiparous	207 (47.4)
Multiparous	229 (52.4)
Missing data	1 (0.2)
Annual household income (million Japanese yen)[b]	
<5	302 (69.1)
≥5	126 (28.8)
Missing data	9 (2.1)
Blood sampling period[b]	
During pregnancy	281 (64.3)
After delivery	156 (35.7)
Maternal serum level[c]	
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[b]	
PPARA (T>C, dbSNP ID: rs1800234)	
TT	390 (89.2)
TC	44 (10.1)
CC	3 (0.7)
TC/CC	47 (10.8)
PPARA (G>A, dbSNP ID: rs135561)	
GG	388 (88.8)
GA	48 (11.0)
AA	1 (0.2)
GA/AA	49 (11.2)
PPARG (C>T, dbSNP ID: rs3856806)	
CC	313 (71.6)
CT	111 (25.4)
TT	13 (3.0)
CT/TT	124 (28.4)
PPARGC1A (C>T, dbSNP ID: rs2970847)	
CC	269 (61.6)
CT	149 (34.1)
TT	19 (4.3)
CT/TT	168 (38.4)
PPARGC1A (G>A, dbSNP ID: rs8192678)	
GG	120 (27.5)
GA	215 (49.2)
AA	102 (23.3)
GA/AA	317 (72.5)
PPARD (T>C, dbSNP ID: rs1053049)	
TT	269 (61.6)
TC	157 (35.9)
CC	11 (2.5)
TC/CC	168 (38.4)
PPARD (A>G, dbSNP ID: rs2267668)	
AA	287 (65.7)

AG	140 (32.0)
GG	10 (2.3)
AG/GG	150 (34.3)
CAR (T>C, dbSNP ID: rs2307424)	
TT	116 (26.5)
TC	235 (53.8)
CC	86 (19.7)
TC/CC	321 (73.5)
CAR (A>G, dbSNP ID: rs2501873)	
AA	139 (31.8)
AG	232 (53.1)
GG	66 (15.1)
AG/GG	298 (68.2)
LXRA (A>G, dbSNP ID: rs2279238)	
AA	184 (42.1)
AG	203 (46.5)
GG	50 (11.4)
AG/GG	253 (57.9)
LXRB (T>C, dbSNP ID: rs1405655)	
TT	287 (65.7)
TC	129 (29.5)
CC	21 (4.8)
TC/CC	150 (34.3)
LXRB (G>A, dbSNP ID: rs2303044)	
GG	297 (68.0)
GA	125 (28.6)
AA	15 (3.4)
GA/AA	140 (32.0)
LXRB (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; *PPARGC1A*, peroxisome proliferator-activated receptor gamma coactivator 1-alpha.

[a] Mean ± Standard deviation (SD).

[b] *n* (%).

[c] Median (inter-quartile range; IQR).

Table 2. Association between MEHP and fatty acid levels

	Exposure: MEHP		
	Crude	Adjusted 1	Adjusted 2
Outcome	β (95% CI)	β (95% CI)	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 3rd 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 $\log_{10}$-transformed PFOS level (ng/mL).
β (95% CI) represents the change in $\log_{10}$-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)
	PPARGC1A-GA/AA	-0.088 (-0.256, 0.081)	-0.080 (-0.249, 0.088)	-0.072 (-0.239, 0.095)
	MEHP × *PPARGC1A*-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)**
	PPARGC1A-GA/AA	-0.111 (-0.236, 0.014)	-0.117 (-0.241, 0.010)	-0.113 (-0.239, 0.014)
	MEHP × *PPARGC1A*-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)
	PPARGC1A-GA/AA	0.039 (-0.147, 0.226)	-0.011 (-0.190, 0.168)	0.050 (-0.136, 0.237)
	MEHP × *PPARGC1A*-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)*
	PPARGC1A-GA/AA	-0.082 (-0.179, 0.014)	-0.097 (-0.215, 0.022)	-0.100 (-0.219, 0.018)
	MEHP × *PPARGC1A*-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)**
	PPARGC1A-GA/AA	-0.144 (-0.285, -0.003)*	-0.146 (-0.288, -0.004)*	-0.141 (-0.283, 0.000)*
	MEHP × *PPARGC1A*-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)
	PPARGC1A-GA/AA	-0.095 (-0.389, 0.199)	-0.105 (-0.403, 0.193)	-0.084 (-0.375, 0.207)
	MEHP × *PPARGC1A*-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)
	PPARGC1A-GA/AA	-0.067 (-0.409, 0.274)	-0.049 (-0.393, 0.295)	-0.031 (-0.370, 0.308)
	MEHP × *PPARGC1A*-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)
	PPARGC1A-GA/AA	0.108 (-0.178, 0.394)	0.100 (-0.191, 0.391)	0.112 (-0.176, 0.401)
	MEHP × *PPARGC1A*-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)
	PPARGC1A-GA/AA	0.040 (-0.266, 0.346)	0.055 (-0.256, 0.365)	0.045 (-0.264, 0.355)
	MEHP × *PPARGC1A*-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)
	PPARGC1A-GA/AA	0.186 (-0.122, 0.493)	0.181 (-0.132, 0.495)	0.187 (-0.127, 0.500)
	MEHP × *PPARGC1A*-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 3rd 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 $\log_{10}$-transformed PFOS level (ng/mL).
β (95% CI) represents the change in $\log_{10}$-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)*
	LXRB-GA/AA	-0.044 (-0.202, 0.114)	-0.075 (-0.232, 0.082)	-0.067 (-0.223, 0.088)
	MEHP × *LXRB*-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)**
	LXRB-GA/AA	-0.069 (-0.186, 0.048)	-0.075 (-0.194, 0.043)	-0.072 (-0.190, 0.047)
	MEHP × *LXRB*-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)**
	LXRB-GA/AA	-0.053 (-0.227, 0.122)	-0.079 (-0.254, 0.095)	-0.074 (-0.247, 0.100)
	MEHP × *LXRB*-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)
	LXRB-GA/AA	0.013 (-0.097, 0.122)	0.010 (-0.100, 0.121)	0.007 (-0.103, 0.118)
	MEHP × *LXRB*-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)**
	LXRB-GA/AA	-0.053 (-0.185, 0.080)	-0.071 (-0.204, 0.062)	-0.067 (-0.200, 0.066)
	MEHP × *LXRB*-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)**
	LXRB-GA/AA	-0.248 (-0.519, 0.024)	-0.268 (-0.543, 0.006)	-0.249 (-0.517, 0.019)
	MEHP × *LXRB*-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)*
	LXRB-GA/AA	-0.114 (-0.432, 0.203)	-0.162 (-0.481, 0.156)	-0.144 (-0.458, 0.170)
	MEHP × *LXRB*-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)
	LXRB-GA/AA	-0.182 (-0.448, 0.083)	-0.188 (-0.457, 0.082)	-0.176 (-0.442, 0.091)
	MEHP × *LXRB*-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)
	LXRB-GA/AA	0.075 (-0.210, 0.361)	0.052 (-0.237, 0.341)	0.042 (-0.246, 0.330)
	MEHP × *LXRB*-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)
	PPARGC1A-GA/AA	-0.114 (-0.400, 0.171)	-0.114 (-0.404, 0.176)	-0.108 (-0.398, 0.181)
	MEHP × *PPARGC1A*-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 3rd 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 $\log_{10}$-transformed PFOS level (ng/mL).
β (95% CI) represents the change in $\log_{10}$-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.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.