Journal of Developmental Origins of Health and Disease

www.cambridge.org/doh

Association between mercury in cord serum and sex-specific DNA methylation in cord tissues

Shino Nishizawa-Jotaki[1], Kenichi Sakurai[2], Akifumi Eguchi[1], Hiromi Tanabe[2], Masahiro Watanabe[1] and Chisato Mori[1,3]

[1]Department of Sustainable Health Science, Center for Preventive Medical Sciences, Chiba University, Chiba, Japan; [2]Department of Nutrition and Metabolic Medicine, Center for Preventive Medical Sciences, Chiba University, Chiba, Japan and [3]Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan

Original Article

Cite this article: Nishizawa-Jotaki S, Sakurai K, Eguchi A, Tanabe H, Watanabe M, and Mori C. (2021) Association between mercury in cord serum and sex-specific DNA methylation in cord tissues. *Journal of Developmental Origins of Health and Disease* **12**: 124–131. doi: 10.1017/S2040174420000161

Received: 12 August 2019
Revised: 12 February 2020
Accepted: 15 February 2020
First published online: 3 April 2020

Keywords:
C-MACH; prenatal exposure; mercury; DNA methylation

Address for correspondence:
Chisato Mori, Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, Chuo-ku Inohana 1-8-1, Chiba, 260-8670, Japan.
Email: cmori@faculty.chiba-u.jp

Abstract

Prenatal exposure to mercury *in utero* causes abnormal foetal growth and adverse outcomes. DNA methylation is currently considered a possible mechanism through which this occurs. However, few studies have investigated the association between prenatal exposure to mercury and DNA methylation in detail. This study aimed to clarify the relationship between prenatal exposure to total mercury (Hg) and DNA methylation and its associations with sex-specific characteristics in male and female offspring. In a birth cohort study known as the Chiba study of Mother and Child Health, the DNA methylation status in cord tissue and Hg concentrations in cord serum were examined. A total of 67 participants (27 males and 40 females) were analysed based on Spearman's correlations, adjusted by a false discovery rate of the sex of each offspring. Only one methylated locus was positively correlated with Hg concentrations in cord serum in male offspring, but not in female offspring, and was annotated to the haloacid dehalogenase-like hydrolase domain-containing protein 1 (*HDHD1*) gene on chromosome X. This locus was located in the intron of the *HDHD1* gene body and is a binding site for the zinc finger protein CCCTC-binding factor. One of the other loci, located in *HDHD1*, was highly methylated in the group with higher mercury concentrations, and this locus was in the gene body of *HDHD1*. Our results suggest that prenatal exposure to Hg might affect the epigenetic status of male foetuses.

Introduction

Mercury is a ubiquitous global environmental contaminant; it exists in elemental, organic and inorganic forms and can be found in humans, plants and animals all around the world. All forms of mercury cause toxicity, such as gastrointestinal toxicity, neurotoxicity and nephrotoxicity; however, methylmercury (MeHg) is one of the most toxic forms of this element.[1,2] Exposure to MeHg typically occurs upon consuming fish and other seafood.[3]

MeHg can be transported from maternal blood circulation to the foetus through the placenta and accumulate in foetal tissues.[4] In addition, the concentration of MeHg is higher in cord tissue than that in the placenta because of the extremely high placental transfer.[5] Several studies have reported that prenatal exposure to MeHg affects foetuses or infants adversely, causing low birth weight and/or neurodevelopmental disorders; moreover, the effects of exposure to MeHg are much stronger in foetuses than in their mothers.[3,6] Prenatal exposure to MeHg, even at low levels, can potentially affect foetal development.[7] Further, it was determined that the total concentration of mercury in the blood is a validated biomarker for estimating MeHg in the general human population[8]; accordingly, the levels of total mercury and MeHg were found to be almost the same in cord blood.[9] Moreover, it has been reported that the total concentration of mercury in the blood can be an indicator of MeHg, and total mercury rather than MeHg has been measured in several epidemiological studies.[7]

Foetal environmental factors, such as intrauterine malnutrition, are considered to be associated with an increased risk of developing heart disease, diabetes, hypertension, stroke, obesity, cancer and many other non-communicable diseases in later life.[10–12] This is known as the 'developmental origins of health and disease' (DOHaD) concept.[13] In addition to nutritional status, it has also been shown that environmental pollutants affect foetuses or children in a DOHaD-dependent manner.

Epigenetic modifications, such as DNA methylation, have been described as potential mechanisms underlying the onset of DOHaD-type effects.[14–16] Prenatal exposure to mercury in cord blood or toenails has reportedly been associated with the DNA methylation of some genes in cord blood cells.[17,18] Recent prospective epidemiological studies have shown that not only high levels but also low levels of prenatal exposure to mercury *in utero* might lead to lower cognitive test scores in children.[7,19]

CAMBRIDGE UNIVERSITY PRESS

Table 1. Characteristics of the study population

	Total ($n = 67$)	Male offspring ($n = 27$)	Female offspring ($n = 40$)	p-Value[a]
Maternal characteristics				
Age (year)	33 (5)	33 (7)	33 (5)	0.6164
Height (cm)	160.0 (7.3)	159.0 (9.7)	160.0 (5.3)	0.9796
Pre-pregnancy weight (kg)	52.0 (8.0)	52.0 (9.1)	52.0 (7.3)	0.5998
Pre-pregnancy BMI (kg/m^2)	20.5 (3.0)	20.0 (3.6)	20.7 (2.5)	0.4741
Offspring characteristics at birth				
Total mercury concentrations in cord serum (ng/g)	0.760 (0.600)	0.900 (0.645)	0.620 (0.473)	0.0787
Weight (g)	3028 (344)	2992 (415)	3029 (312)	0.9949
Height (cm)	49.0 (2.0)	49.0 (1.8)	49.0 (2.0)	0.5078
Head circumference (cm)	33.0 (1.0)	33.0 (1.3)	33.0 (1.5)	0.3479

Values are shown as median (IQR, interquartile range).
[a]Mann–Whitney *U*-test, comparing male and female offspring.

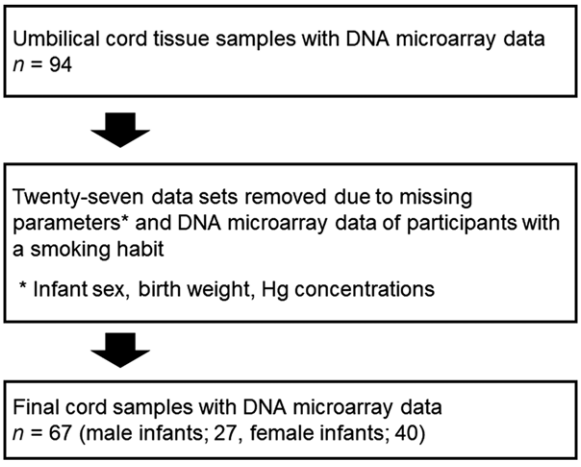

Fig. 1. Final study population for DNA methylation microarray analysis; 94 umbilical cord samples were assayed on the Infinium Methylation Epic BeadChip, and 67 samples were analysed for correlation between Hg concentration in cord serum and DNA methylation status in the cord tissue.

It has been shown that prenatal exposure to environmental chemicals produces sex-specific differences; the effects of a mother's passive smoking on the offspring have already been recognised.[20,21] Furthermore, prenatal exposure to heavy metals such as cadmium or arsenic might alter patterns of DNA methylation in infants, and it has been shown that there are sex-specific associations between exposure to heavy metals and DNA methylation.[22,23] Further, some studies in rats[24] and humans[25] have reported that during development, males are more sensitive to mercury exposure than females. However, it remains unclear whether males or females are affected differently by MeHg[26]; thus, the results regarding a sex-specific response to mercury are controversial. The association between exposure to mercury and the status of foetal DNA methylation and its sex-specific effects have not been thoroughly evaluated; therefore, it is necessary to determine the sex-specific effects of prenatal exposure to mercury. A recent investigation demonstrated that umbilical cord tissue is a useful epigenetic surrogate that can reflect the effects of exposure to environmental substances during gestation.[27] Accordingly, we used umbilical cord tissue for our DNA methylation analysis.

It has also been reported that selenium (Se) could weaken the harmful effects of mercury in toenails.[28] Moreover, Se might alter foetal growth restrictions related to MeHg exposure in cord blood[29]; however, the effects of Se on mercury-induced DNA methylation have not been evaluated yet. In this study, we separately analysed male and female offspring using DNA methylation array analysis to elucidate the relationship between the concentration of total mercury (Hg) in umbilical cord serum and the status of DNA methylation in umbilical cord tissue (as a part of the foetus). Furthermore, we determined sex-specific patterns of DNA methylation and the effects of Se on prenatal Hg-induced DNA methylation.

Methods

Study population

The Chiba Study of Mother and Child Health (C-MACH) is a three-hospital-based birth cohort study, including approximately 400 mother–child pairs, and it was used to investigate the association between prenatal exposure to Hg and epigenetic changes in offspring in this study. Participants were recruited between February 2014 and June 2015.[30] Clinical maternal information during pregnancy was collected at 12 and 32 weeks of gestation. Clinical information regarding the newborn offspring was collected at birth.

Ninety-four participants from the C-MACH cohort study at the Onodera Ladies Clinic in Chiba were included in the present study because their umbilical cord quality was considered appropriate for DNA methylation array analysis. We did not include participants who had a smoking habit, as it is known to affect the epigenetic status, and we also excluded patients with missing data (offspring sex, Hg concentrations, and birth weight or DNA microarray data). In the end, we used 67 data sets (male = 27, female = 40) for our analysis (Fig. 1). Clinical data regarding the mothers and their offspring included in this study are shown in Table 1.

https://doi.org/10.1017/S2040174420000161 Published online by Cambridge University Press

Sample collection and Hg and Se measurements

Blood from the umbilical cord was collected at childbirth and subsequently centrifuged to isolate serum; umbilical cord tissue was collected simultaneously. These samples were submitted to cold storage and immediately transported from the clinic to the laboratory. After we washed the umbilical cord tissues at the laboratory, we stored the samples at −80 °C until further analysis.

Since whole blood samples were not obtained in this study, serum samples were used to measure Hg. The concentration of Hg was determined at Shimadzu Techno-Research Inc. (Kyoto, Japan) using inductively coupled plasma-mass spectrometry (ICP-MS) (Agilent Technologies, Tokyo, Japan). A detailed protocol is described elsewhere.[31] Briefly, serum samples containing a volume of 0.5 ml were diluted (1:19) with the dilution solution (2% v/v 1-butanol, 0.1% Tetramethylammonium hydroxide, 0.05% polyoxyethylene (10) octyl phenyl ether and 0.05% w/v ethylenediamine tetraacetic acid), mixed by vortexing and analysed by ICP-MS. In addition, the concentration of Se, as well as the concentration of Hg in serum, was measured. The detection limits for Hg and Se were 0.050 and 1.7 ng g^{-1}, respectively.

DNA methylation analysis

The methylation status of genomic DNA in the cord tissue was analysed as previously described.[32] Briefly, genomic DNA was extracted from cord tissues using the NucleoSpin Tissue Kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) and bisulphite conversion was performed using the EZ DNA Methylation Gold Kit (Zymo Research, Los Angeles, CA, USA). Bisulphite-converted DNA was applied to the Infinium Methylation EPIC BeadChip (Illumina, San Diego, CA, USA). Infinium Methylation EPIC BeadChip profiles the methylation status of over 850,000 CpG sites at single-nucleotide resolution; the BeadChips were scanned with an Illumina iScan (Illumina) array scanner. Data were analysed using the Illumina GenomeStudio software (Illumina). GenomeStudio® provides average β values, which indicate methylation levels at each CpG site (1 = fully methylated, 0 = fully unmethylated). According to Illumina users guide (GenomeStudio® Methylation Module v1.8 User Guide), data normalisation, background subtraction and colour channel normalisation were performed. Raw data were normalised using the ratio of intensities between methylated and unmethylated alleles, and background was subtracted using built-in negative control bead types. We monitored the quality of methylation experiments by checking built-in internal quality controls in the Controls Dashboard of the methylation module of GenomeStudio®. For all samples, it was confirmed that the number of detected CpGs (p-value = 0.05) were at least >96%. Probes without calculated β values and exhibiting low variance (standard deviation < 0.1) were initially excluded from the analysis.[33] Probes located on chromosome Y were also excluded from female offspring analysis. The final data set included 15,071 probes in male offspring and 14,678 probes in female offspring.

Data analysis

Statistical analyses were performed using R v3.4.4[34] (https://www.r-project.org/). The characteristics of each sex were compared using a Mann–Whitney U-test. Correlations between β values and Hg concentrations were analysed by Spearman's rank correlation in male and female offspring, separately. Adjustment for multiple testing was performed with a false discovery rate (FDR) calculated by the R package psych.[35,36] An FDR value < 0.05 was defined as statistically significant. In addition, the correlation between birth weight and Hg concentrations was also analysed by Spearman's rank test. Next, multivariate regression analyses were performed: Model 1, adjusted for maternal age and birth weight; Model 2, adjusted for maternal age, birth weight and the concentration of Se in cord serum; Model 3, adjusted for maternal age, gestational age and the concentration of Se in cord serum. Since the birth weight and the gestational age were related, the birth weight was removed from Model 3 to reduce multicollinearity. Cell-type proportion (stromal cell, endothelial cell and epithelial cell) was estimated using the R/Bioconductor packages minfi.[37,38] Multiple regression analyses were performed using endothelial cell ratio and epithelial cell ratio as explanatory variables (Model 4) after confirming the multicollinearity of each cell ratio in addition to Model 1. In some cases, the concentration of Hg was dichotomised into 'higher concentration' and 'lower concentration' groups based on the median value of Hg (0.9 ng/g serum). Moreover, we performed an additional Mann–Whitney U-test to measure and compare correlations between β value and Hg concentration for the probes of interest between these two groups. The additional analysis validated the previous results.

Results

Characteristics of mothers and their offspring

After excluding all participants with smoking habits and/or missing parameters, 67 participants were included in this study. Characteristics of mothers and offspring are shown in Table 1. The median values and the interquartile range (IQR) values (in parenthesis) of maternal age, height, pre-pregnancy weight and pre-pregnancy body mass index were 33 (5) years, 160.0 (7.3) cm, 52.0 (8.0) kg and 20.5 (3.0) kg/m^2, respectively. The medians and IQRs (in parenthesis) of offspring weight, height and head circumference at birth were 3028 (344), 49.0 (2.0) and 33.0 (1.0), respectively. For each parameter, a few offspring deviated by more than two standard deviations, but the majority were within the standard range. The characteristics of male offspring were comparable to those of female offspring. No significant correlation between birth weight and cord Hg was observed.

DNA methylation in the umbilical cord and Hg concentration in cord serum

Fig. 2 shows a Manhattan plot of all probes included in this analysis. There was one probe on chromosome X (cg02027844) that significantly correlated with the concentration of Hg in the cord serum. The β value of cg02027844 was positively correlated with Hg concentrations in cord serum of males (Fig. 3a, $r = 0.767$, $p = 3.06$ E-06, $q = 0.046$). It has been previously reported that maternal age[39] and birth weight[40] affect DNA methylation in the umbilical cord. Therefore, we estimated the association between methylation status and the concentration of Hg in cord serum using multiple regression analysis for maternal age, birth weight and gestational age, which could be confounders. In addition, multiple regression analyses were also performed using estimated cell-type proportions. As shown in Table 2, after adjusting for covariates, the correlation between methylation status and Hg concentration was still significant. Moreover, the β value was significantly higher ($p < 0.01$) in the group with higher concentrations of Hg (mean β value; 0.59) than that in the group with lower concentrations of Hg (mean β value; 0.50) in the

https://doi.org/10.1017/S2040174420000161 Published online by Cambridge University Press

Table 2. Multivariate-adjusted coefficients of Hg concentrations in cord serum according to the methylation status of the cord tissue

	Coefficients	Standard error of coefficients	*p*-Value
Model 1	0.076	0.028	0.013
Model 2	0.073	0.029	0.020
Model 3	0.066	0.028	0.024
Model 4	0.073	0.030	0.024

Model 1: Adjusted by maternal age and birth weight.
Model 2: Adjusted by maternal age, birth weight and Se concentration in cord serum.
Model 3: Adjusted by maternal age, gestational age and Se concentration in cord serum.
Model 4: Adjusted by maternal age, birth weight, endothelial ratio and epithelial ratio.

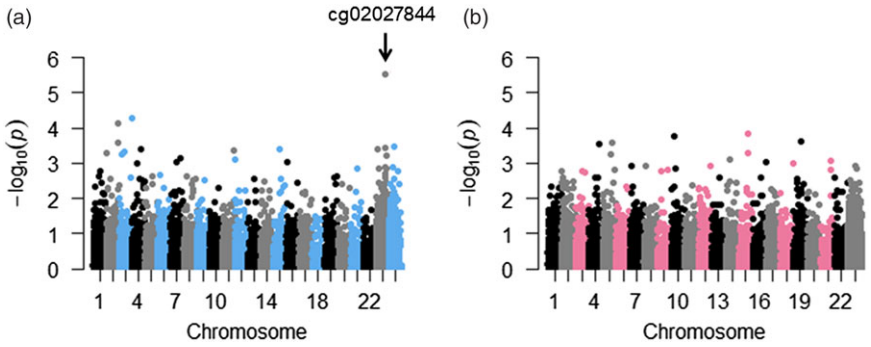

Fig. 2. Manhattan plots for mercury concentration in cord serum and methylation status in cord tissue (a) in males and (b) females.

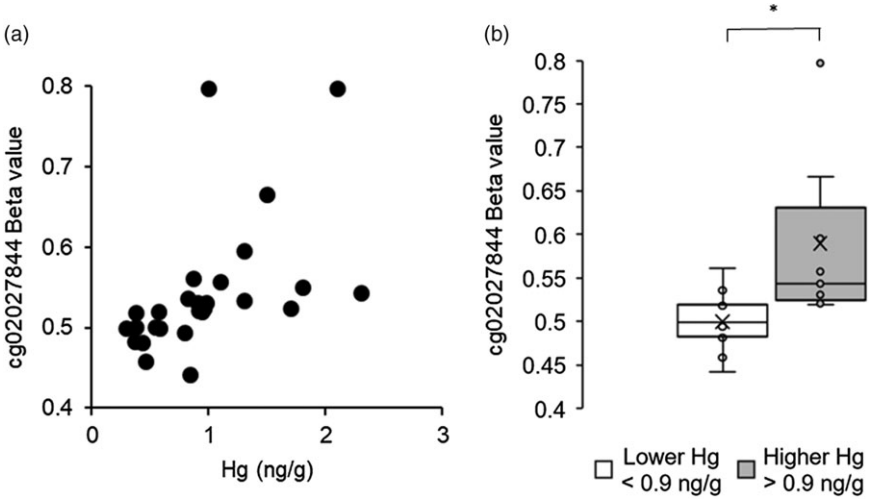

Fig. 3. (a) Correlation between cg02027844 methylation (β value) and Hg concentrations in cord blood (ng/g) in males, (FDR = 0.046); (b) cg02027844 methylation (β value) comparing lower ($n = 13$) and higher ($n = 13$) Hg concentration groups in males, *: $p < 0.01$. 'X' indicates mean values.

Mann–Whitney *U*-test (Fig. 3b). In females, no correlation between the concentration of Hg and the status of DNA methylation was detected based on any of the probes. The β values of cg02027844 in female offspring, which significantly correlated with Hg concentrations in male offspring, are shown in Supplementary Figure S1 ($r = 0.033$, $p = 0.837$, $q = 0.999$).

The methylation of probe cg02027844 was annotated to the haloacid dehalogenase-like hydrolase domain-containing protein 1 (*HDHD1*) gene. This sequence is located in intron 4 of *HDHD1* (https://genome.ucsc.edu/). Two other probes were annotated to *HDHD1*, namely cg03572700 and cg18204732. The β value of cg18204732, but not cg03572700, was significantly greater ($p < 0.01$) in the higher Hg concentration group (mean β value; 0.73) than in the lower Hg concentration group (mean β value; 0.42) (Fig. 4). Probe cg18204732 pertains to a sequence located in intron 1 of the gene body of *HDHD1*.

https://doi.org/10.1017/S2040174420000161 Published online by Cambridge University Press

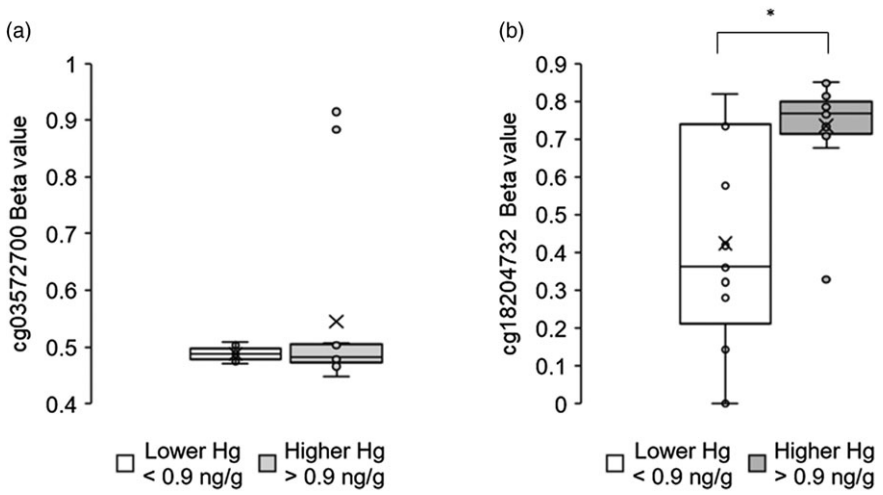

Fig. 4. Comparison of β values at the methylation sites annotated to *HDHD1* and the Hg concentrations in cord serum (ng/g) in males (a; cg03572700, b; cg18204732, FDR = 0.99). *: $p < 0.01$. 'X' indicates mean values.

Effects of Se on DNA methylation

No significant correlation was observed between the concentrations of Hg and Se in this study. To consider whether Se interferes with the effect of Hg on DNA methylation in umbilical cords, multiple regression analysis was carried out with the addition of cord serum Se levels to Model 1. However, as a result, the correlation between the status of DNA methylation and the concentration of Hg was still significant (Table 2; Model 2).

Discussion

In this exploratory study of 67 Japanese infants, serum mercury concentrations at birth were associated with the DNA methylation of a single CpG site in cord tissue obtained from male infants only. This CpG site was annotated to the *HDHD1* gene on chromosome X. In female newborns, no associations were found between the concentration of mercury in cord serum and the status of DNA methylation in cord tissue. Overall, the concentration of mercury in the serum was low, and only one probe was correlated with DNA methylation in this study. However, several studies have reported that prenatal exposure to mercury changes DNA methylation patterns in offspring and is associated with adverse neurobehavioural outcomes.[41,42] Furthermore, recent prospective epidemiological studies have shown that prenatal exposure to mercury *in utero*, even at low concentrations, might lead to lower cognitive test scores in children.[7] These results suggest that prenatal exposure to mercury even at low concentrations can affect postnatal development in offspring via DNA methylation.

In this study, no correlation was found between the concentration of mercury and DNA methylation status in cord tissue samples taken from females. Although the reason for this sex-specific difference is unclear, no statistically significant difference was observed in cord serum between male and female offspring regarding the concentration of mercury. Prenatal exposure to mercury affects neonate behavioural ability and birth weight in males, but no apparent effect was previously observed in females[6,43]; moreover, a decrease in male, but not female, birth rate caused by Minamata disease was also observed.[3] It has been reported that male foetuses/infants are more sensitive to mercury exposure than females during development.[24,25] The protective effect of female hormones against oxidative stress, as well as inflammation, may explain this observation.[26] In fact, MeHg toxicity is related to free radicals and oxidative stress[44] and affects DNA methylation.[45] Therefore, sex-dependent differences between mercury exposure and DNA methylation found in this study may be due to the suppression of mercury-induced oxidative stress via hormonal effects in female offspring. Additionally, prenatal mercury exposure can affect sex-specific DNA methylation of the differentially methylated regions of the paraoxonase 1 gene (*PON1*). This methylation pattern persists throughout childhood and is associated with cognitive performance in males.[41] It has also been reported that DNA methylation levels in monozygotic twins differ between males and females, indicating the possibility that environmental factors can cause sex-specific differences.[46] Therefore, male but not female offspring may be susceptible to the effects of prenatal mercury exposure.

The cg02027844 locus was annotated to the *HDHD1*, which is located on chromosome X at band Xp22.31 and encodes pseudouridine-5′-phosphatase (PUDP). *HDHD1* is widely expressed not only in organs, such as the placenta and the colon, but also in lymphocytes.[47] Although the biological function of this gene has not been thoroughly investigated yet, *HDHD1* might be involved in RNA processing and turnover.[48] Although DNA methylation of a promoter region suppresses gene expression, DNA methylation of a gene body is thought to be involved in controlling splicing.[49,50] Site cg02027844 is located in intron 4, in the gene body of *HDHD1*; therefore, the methylation of this locus might control the splicing but not the expression of this gene. Besides, locus cg02027844 corresponds to a binding site for the zinc finger protein CCCTC-binding factor (CTCF, https://genome.ucsc.edu/), which is considered essential for insulator activity.[51] One of the known functions of an insulator is to separate enhancers and promoters to block their interactions.[52] CTCF binding to a subset of DNA sequences can be disrupted by DNA methylation.[53] Consequently, the functions of an insulator might be suppressed by disrupting CTCF binding due to DNA methylation. Although we could not examine the expression of *HDHD1* or mRNA splicing due to circumstances associated with sample collection, DNA methylation at the CTCF-binding site caused by prenatal exposure to mercury could potentially affect the mRNA splicing of *HDHD1* or nearby genes. Two other probes

https://doi.org/10.1017/S2040174420000161 Published online by Cambridge University Press

were annotated to *HDHD1*, namely cg03572700 and cg18204732; the β value of cg18204732 was significantly greater in the group with higher concentrations of mercury than in the group with lower mercury concentrations. Further, the results of probe cg02027844 might support the possibility that prenatal exposure to mercury affects the epigenetic status of *HDHD1* via DNA methylation. The scheme of the location of probes in *HDHD1* is shown in Supplementary Figure S2. As a study of effect size, multiple regression analyses were performed in the same model, as shown in Table 2, using standardising objective variables and all explanatory variables. It was shown that the effect of mercury concentration on cg02027844 β value in any model was larger than other explanatory variables (Supplementary Table S2).

In humans, *HDHD1* might be associated with Crohn's disease since PUDP (encoded by *HDHD1*) is associated with its progression.[54] *HDHD1* might also be associated with X-linked ichthyosis, a severe skin disease. Moreover, *HDHD1* is often deleted along with the steroid sulfatase (*STS*) gene in patients with X-linked ichthyosis. *STS* is considered responsible for X-linked ichthyosis[47]; however, no effects were observed on DNA methylation using any of the probes annotated to *STS* in this study. Furthermore, the absence of PUDP activity might contribute to the development of testicular cancer and cryptorchidism.[55] Follow-up studies would be required to clarify whether prenatal epigenetic changes to this gene are associated with the pathophysiology of these diseases. Also, microRNA 4767 (mir4767) is located in the *HDHD1* gene region. Since miR-144 is a microRNA that contributes to inflammation during atopic dermatitis via activation of the NF-kB pathway in the cord serum of infants,[56] changes to the DNA methylation of cg02027844 might affect the expression of mir4767. Further study would be required to follow the health conditions of infants in later life.

The mean β values for each probe on *HDHD1* were 0.51–0.59 and 0.76–0.86 in male and female offspring, respectively (Supplementary Table S1). A previous study by Sharp *et al.*[57] described that *HDHD1* is one of the genes for escaping X chromosome inactivation (XCI). Moreover, DNA methylation levels are reportedly not high in the escaping genes, and the difference in DNA methylation levels between cells with one X chromosome and two X chromosomes is small. Here, although DNA methylation levels were not low (0.51–0.59 and 0.76–0.86 in male and female offspring, respectively), our results are generally consistent with the previous report as the difference in male and female offspring is relatively small. This study does not reveal the relationship between the fact that this gene escapes from XCI in female offspring and that the effects of mercury exposure were observed only in male offspring. The relationship between the effects of environmental pollutants, including mercury, on genes in the X chromosome and XCI status and the differences in their effects depending on sex demand further investigation.

A previous report found an association between prenatal exposure to mercury, including MeHg and the DNA methylation of differentially methylated regions in *PON1* and transcription elongation factor A N-terminal and central domain containing 2 (*TCEANC2*) in umbilical cord blood.[17,41] These genes were present on the EPIC BeadChip used in this study. However, since none of the standard deviations associated with β values of probes annotated to these genes was 0.1 or more, no correlation analysis between mercury concentrations and DNA methylation was performed. That is, the lack of an association between DNA methylation status of these genes and mercury concentrations in this study is likely due to differences in sample types and the small sample size. It has been reported that Se can suppress the toxic effects of exposure to mercury in humans.[28] One of the mechanisms of MeHg toxicity is related to free radicals and oxidative stress[44]; however, Se, an antioxidant, might protect the organism from MeHg toxicity, as shown in a previous *in vitro* experiment.[58] As a result of multiple regression analysis, Se did not contribute to the relationship between DNA methylation and mercury concentration in this study. The narrow range of Se concentrations likely caused this lack of association. Further studies are needed to verify the role of Se in mercury-induced DNA methylation.

There were several limitations to this study. The sample size was relatively small, and the statistical power was not sufficient to analyse all probes. The role of the methylated cg02027844 locus was not clarified because *HDHD1* expression was not examined in this study. Moreover, we cannot entirely exclude confounding factors. Further experiments with a much larger sample size that consider potential confounding factors, including cell types of cord tissue, might be needed to replicate and extend the findings observed in this study.

This is the first study to report the potential association between prenatal exposure to mercury in cord serum and the status of DNA methylation in cord tissue and its sex-specific effects. The level of DNA methylation within *HDHD1* was significantly correlated with mercury concentrations in cord serum obtained from male offspring. This CpG site is located in the CTCF-binding site, which can affect gene expression. These findings suggest that prenatal exposure to mercury can affect the epigenetic status of a foetus. Further studies with larger sample sizes to determine the levels of *HDHD1* gene products are required for clarifying the effects of prenatal mercury exposure on epigenetic alterations as well as health and disease in later life.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S2040174420000161

Acknowledgements. The authors appreciate all the study participants and members of the C-MACH group. The authors thank Professor Hideoki Fukuoka (Fukushima Medical University/Waseda University) for his contribution to the planning and conducting C-MACH. The authors would like to thank Editage (www.editage.com) for English language editing.

Financial Support. This study was supported by a JSPS KAKENHI (grant Number 16H01781, Grant-in-Aid for Scientific Research A, and 16K12608, Grant-in-Aid for Challenging Exploratory Research) from the Japan Society for the Promotion of Science, and Chiba Foundation for Health Promotion & Disease Prevention. The funders had no role in the study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest. None.

Ethical Standards. This study was approved by the Biomedical Research Ethics Committee of the Graduate School of Medicine, Chiba University (ID: 941). Written informed consent was obtained from each study participant.

References

1. Kimáková T, Kuzmová L, Nevolná Z, Bencko V. Fish and fish products as risk factors of mercury exposure. *Ann Agric Environ Med.* 2018; 25, 488–493.
2. Rice KM, Walker EM, Wu M, Gillette C, Blough ER. Environmental mercury and its toxic effects. *J Prev Med Public Health.* 2014; 47, 74–83.
3. Sakamoto M, Tatsuta N, Izumo K, *et al.* Health Impacts and Biomarkers of Prenatal Exposure to Methylmercury: Lessons from Minamata, Japan. *Toxics.* 2018; 6, 45.

4. Stern AH, Smith AE. An assessment of the cord blood: Maternal blood methylmercury ratio: Implications for risk assessment. *Environ Health Perspect.* 2003; 111, 1465–1470.
5. Sakamoto M, Yasutake A, Domingo JL, *et al.* Relationships between trace element concentrations in chorionic tissue of placenta and umbilical cord tissue: Potential use as indicators for prenatal exposure. *Environ Int.* 2013; 60, 106–111.
6. Tatsuta N, Kurokawa N, Nakai K, *et al.* Effects of intrauterine exposures to polychlorinated biphenyls, methylmercury, and lead on birth weight in Japanese male and female newborns. *Environ Health Prev Med.* 2017; 22, 39.
7. Karagas MR, Choi AL, Oken E, *et al.* Evidence on the human health effects of low-level methylmercury exposure. *Environ Health Perspect.* 2012; 120, 799–806.
8. Sheehan MC, Burke TA, Navas-Acien A, *et al.* Global methylmercury exposure from seafood consumption and risk of developmental neurotoxicity: a systematic review. *Bull World Health Organ.* 2014; 92, 254–269F.
9. Iwai-Shimada M, Kameo S, Nakai K, *et al.* Exposure profile of mercury, lead, cadmium, arsenic, antimony, copper, selenium and zinc in maternal blood, cord blood and placenta: The Tohoku Study of Child Development in Japan. *Environ Health Prev Med.* 2019; 24, 35.
10. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet.* 1989; 2, 577–580.
11. Roseboom T, de Rooij S, Painter R. The Dutch famine and its long-term consequences for adult health. *Early Hum Dev.* 2006; 82, 485–491.
12. De Boo HA, Harding JE. The developmental origins of adult disease (Barker) hypothesis. *Aust N Z J Obstet Gynaecol.* 2006; 46, 4–14.
13. Gluckman PD, Hanson MA. Living with the past: Evolution, development, and patterns of disease. *Science.* 2004; 17, 1733–1736.
14. Bianco-Miotto T, Craig JM, Gasser YP, Van Dijk SJ, Ozanne SE. Epigenetics and DOHaD: From basics to birth and beyond. *J Dev Orig Health Dis.* 2017; 8, 513–519.
15. Jones MJ, Goodman SJ, Kobor MS. DNA methylation and healthy human aging. *Aging Cell.* 2015; 14, 924–932.
16. Eguchi A, Nishizawa-Jotaki S, Tanabe H, *et al.* An Altered DNA Methylation Status in the Human Umbilical Cord Is Correlated with Maternal Exposure to Polychlorinated Biphenyls. *Int J Environ Res Public Health*. Epub ahead of print 2019. DOI: 10.3390/ijerph16152786.
17. Bakulski KM, Lee HJ, Feinberg JI, *et al.* Prenatal mercury concentration is associated with changes in DNA methylation at TCEANC2 in newborns. *Int J Epidemiol.* 2015; 44, 1249–1262.
18. Cardenas A, Koestler DC, Houseman EA, *et al.* Differential DNA methylation in umbilical cord blood of infants exposed to mercury and arsenic in utero. *Epigenetics.* 2015; 10, 508–515.
19. Oken E, Wright RO, Kleinman KP, *et al.* Maternal fish consumption, hair mercury, and infant cognition in a U.S. cohort. *Environ Health Perspect.* 2005; 113, 1376–1380.
20. Kishi R, Kobayashi S, Ikeno T, *et al.* Ten years of progress in the Hokkaido birth cohort study on environment and children's health: Cohort profile - Updated 2013. *Environ Health Prev Med.* 2013; 18, 429–450.
21. Veiga-Lopez A, Kannan K, Liao C, *et al.* Gender-specific effects on gestational length and birth weight by early pregnancy BPA exposure. *J Clin Endocrinol Metab*. Epub ahead of print 2015. DOI: 10.1210/jc.2015-1724.
22. Broberg K, Ahmed S, Engström K, *et al.* Arsenic exposure in early pregnancy alters genome-wide DNA methylation in cord blood, particularly in boys. *J Dev Orig Health Dis.* 2014; 5, 288–298.
23. Kippler M, Engström K, Mlakar SJ, *et al.* Sex-specific effects of early life cadmium exposure on DNA methylation and implications for birth weight. *Epigenetics.* 2013; 8, 494–503.
24. Khan A, Sulkowski ZL, Chen T, Zavacki AM, Sajdel-Sulkowska EM. Sex-dependent changes in cerebellar thyroid hormone-dependent gene expression following perinatal exposure to thimerosal in rats. *J Physiol Pharmacol.* 2012; 63, 277–283.
25. Woods JS, Heyer NJ, Russo JE, Martin MD, Farin FM. Genetic polymorphisms affecting susceptibility to mercury neurotoxicity in children: Summary findings from the Casa Pia Children's Amalgam Clinical Trial. *Neurotoxicology.* 2014; 44, 288–302.
26. Kern JK, Geier DA, Homme KG, *et al.* Developmental neurotoxicants and the vulnerable male brain: A systematic review of suspected neurotoxicants that disproportionally affect males. *Acta Neurobiol Exp (Wars).* 2017; 77, 269–296.
27. Sakurai K, Shioda K, Eguchi A, *et al.* DNA methylome of human neonatal umbilical cord: Enrichment of differentially methylated regions compared to umbilical cord blood DNA at transcription factor genes involved in body patterning and effects of maternal folate deficiency or children's sex. *PLoS One.* 2019; 14, e0214307.
28. Park K, Seo E. Toenail mercury and dyslipidemia: Interaction with selenium. *J Trace Elem Med Biol.* 2017; 39, 43–49.
29. Wells EM, Herbstman JB, Lin YH, *et al.* Cord blood methylmercury and fetal growth outcomes in baltimore newborns: Potential confounding and effect modification by omega-3 fatty acids, selenium, and sex. *Environ Health Perspect.* 2016; 124, 373–379.
30. Sakurai K, Miyaso H, Eguchi A, *et al.* Chiba study of mother and children's health (C-MACH): Cohort study with omics analyses. *BMJ Open.* 2016; 6, e010531
31. Eguchi A, Yanase K, Yamamoto M, *et al.* The relationship of maternal PCB, toxic, and essential trace element exposure levels with birth weight and head circumference in Chiba, Japan. *Environ Sci Pollut Res Int.* 2019; 26, 15677–15684.
32. Tachibana K, Sakurai K, Watanabe M, Miyaso H, Mori C. Associations between changes in the maternal gut microbiome and differentially methylated regions of diabetes-associated genes in fetuses: A pilot study from a birth cohort study. *J Diabetes Investig.* 2017; 8, 550–553.
33. Kulis M, Heath S, Bibikova M, *et al.* Epigenomic analysis detects widespread gene-body DNA hypomethylation in chronic lymphocytic leukemia. *Nat Genet.* 2012; 44, 1236–1242.
34. R Core Team (2018). R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing, Vienna, Austria*. URL https://www.R-project.org/.
35. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc*. Epub ahead of print 1995. DOI: 10.2307/2346101.
36. Revelle MW. psych: Procedures for personality and psychological research (R package). https://CRAN.R-project.org/package=psych.
37. Aryee MJ, Jaffe AE, Corrada-bravo H, *et al.* Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics*. 2014; 30, 1363–1369.
38. Lin X, Tan JYL, Teh AL, *et al.* Cell type-specific DNA methylation in neonatal cord tissue and cord blood: a 850K-reference panel and comparison of cell types. *Epigenetics.* 2018; 13, 941–958.
39. Miyaso H, Sakurai K, Takase S, *et al.* The methylation levels of the H19 differentially methylated region in human umbilical cords reflect newborn parameters and changes by maternal environmental factors during early pregnancy. *Environ Res.* 2017; 157, 1–8.
40. Mao LL, Xiao XH, Zhang Q, *et al.* DNA Methylation and Birth Weight: a Genome-wide Analysis. *Biomed Environ Sci.* 2017; 30, 667–670.
41. Cardenas A, Rifas-Shiman SL, Agha G, *et al.* Persistent DNA methylation changes associated with prenatal mercury exposure and cognitive performance during childhood. *Sci Rep.* 2017; 7, 288.
42. Maccani JZ, Koestler DC, Lester B, *et al.* Placental dna methylation related to both infant toenail mercury and adverse neurobehavioral outcomes. *Environ Health Perspect.* 2015; 123, 723–729.
43. Gao Y, Yan CH, Tian Y, *et al.* Prenatal exposure to mercury and neurobehavioral development of neonates in Zhoushan City, China. *Environ Res.* 2007; 105, 390–399.
44. Crespo-López ME, Macêdo GL, Pereira SI, *et al.* Mercury and human genotoxicity: Critical considerations and possible molecular mechanisms. *Pharmacol Res.* 2009; 60, 212–220.
45. Li L, Ling Z, Dong W, *et al.* Dnmt3a-Mediated DNA Methylation Changes Regulate Osteogenic Differentiation of hMSCs Cultivated in the 3D Scaffolds under Oxidative Stress. *Oxid Med Cell Longev*. Epub ahead of print 2019. DOI: 10.1155/2019/4824209.
46. Watanabe M, Honda C, Iwatani Y. Within-pair differences of DNA methylation levels between monozygotic twins are different between male and female pairs. *BMC Med Genomics.* 2016; 9, 55.

https://doi.org/10.1017/S2040174420000161 Published online by Cambridge University Press

47. Fagerberg L, Hallström BM, Oksvold P, *et al.* Analysis of the Human Tissue-specific Expression by Genome-wide Integration of Transcriptomics and Antibody-based Proteomics. *Mol Cell Proteomics.* 2013; 13, 397–406.
48. Preumont A, Rzem R, Vertommen D, Van Schaftingen E. HDHD1, which is often deleted in X-linked ichthyosis, encodes a pseudouridine-5′-phosphatase. *Biochem J.* 2010; 431, 237–244.
49. Maunakea AK, Chepelev I, Cui K, Zhao K. Intragenic DNA methylation modulates alternative splicing by recruiting MeCP2 to promote exon recognition. *Cell Res.* 2013; 23, 1256–1269.
50. Jones PA. Functions of DNA methylation: Islands, start sites, gene bodies and beyond. *Nat Rev Genet.* 2012; 13, 484–492.
51. Ishihara K, Oshimura M, Nakao M. CTCF-Dependent Chromatin Insulator Is Linked to Epigenetic Remodeling. *Mol Cell.* 2006; 23, 733–742.
52. Gaszner M, Felsenfeld G. Insulators: Exploiting transcriptional and epigenetic mechanisms. *Nat Rev Genet.* 2006; 7, 703–713.
53. Kazanets A, Shorstova T, Hilmi K, Marques M, Witcher M. Epigenetic silencing of tumor suppressor genes: Paradigms, puzzles, and potential. *Biochim Biophys Acta - Rev Cancer.* 2016; 1865, 275–288.
54. Drobež CP, Repnik K, Gorenjak M, *et al.* DNA polymorphisms predict time to progression from uncomplicated to complicated Crohn's disease. *Eur J Gastroenterol Hepatol.* 2018; 30, 447–455.
55. Idkowiak J, Taylor AE, Subtil S, *et al.* Steroid sulfatase deficiency and androgen activation before and after puberty. *J Clin Endocrinol Metab.* 2016; 101, 2545–2553.
56. Dissanayake E, Inoue Y, Ochiai S, *et al.* Hsa-mir-144-3p expression is increased in umbilical cord serum of infants with atopic dermatitis. *J Allergy Clin Immunol.* 2019; 143, 447–450.e11.
57. Sharp AJ, Stathaki E, Migliavacca E, *et al.* DNA methylation profiles of human active and inactive X chromosomes. *Genome Res.* 2011; 21, 1592–1600.
58. Tapiero H, Townsend DM, Tew KD. The antioxidant role of selenium and seleno-compounds. *Biomed Pharmacother.* 2003; 57, 134–144.

https://doi.org/10.1017/S2040174420000161 Published online by Cambridge University Press