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分担研究報告書

胎児期ビスフェノール A（BPA）曝露の臍帯血 DNA エピゲノム網羅的メチル化析
An epigenome-wide study of cord blood DNA methylations in relation to prenatal bisphenol A exposure

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研究要旨

Background: Exposure to bisphenol A (BPA) *in utero* is associated with adverse health outcome of the offspring. Differential DNA methylation at specific CpG sites may link BPA exposure to health impacts.

Objective: To investigate cord blood DNA methylation changes associated with prenatal exposure to BPA.

Methods: We assessed DNA methylation in cord blood samples from 277 mother-child pairs from the Sapporo cohort of the Hokkaido Study using the *Illumina HumanMethylation 450 BeadChip*.

Results: We observed that a large portion of BPA-associated differentially methylated CpGs with *p*-value <0.0001 was hypomethylated among all newborns (91%) and female infants (98%), as opposed to hypermethylation (88%) among males. We found 27 and 16 CpGs with a false discovery rate (FDR) <0.05 in the analyses for males and females, respectively. Genes annotated to FDR-corrected CpGs clustered into an interconnected genetic network among males, while they rarely exhibited any interactions in females. In contrast, none of the enrichment for gene ontology (GO) terms with FDR <0.05 was observed for genes annotated to the male-specific CpGs with p <0.0001, whereas the female-specific genes were significantly enriched for GO terms related to cell adhesion.

Conclusion: Our epigenome-wide analysis of cord blood DNA methylations implies potential sex-specific epigenome responses to BPA exposure.

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A．研究目的

Bisphenol A (BPA) is a chemical widely used in consumer products, including plastics, dental sealants, food containers, and thermal receipts (Vandenberg *et al*. 2007). Biomonitoring studies reported BPA was

detectable in the dust, air particles, and water (Asimakopoulos *et al.* 2012). Humans are exposed to this compound through their diet, inhalation of house dust, and skin contact (Vandenberg *et al*. 2012). As a result, BPA is widely found in different populations including children and pregnant women (Covaci *et al*. 2015; Yamamoto *et al*. 2016). BPA is a known endocrine-disrupting chemical (EDC). Due to its widespread human exposure and endocrine-disrupting effects, developmental BPA exposure could have adverse effects on human health. Epidemiological studies have shown that prenatal BPA exposures are associated with various health outcomes including altering birth size (Chou *et al*. 2011; Philippat *et al*. 2012), disruption of hormone balance (Minatoya *et al*. 2017a; Romano *et al*. 2015), obesity (Harley *et al*. 2013; Vafeiadi *et al*. 2016), immune function impairment (Gascon *et al*. *2015*; Spanier *et al*. *2014*), and neurobehavioral problems (Braun *et al*. 2017a; Braun *et al*. 2017b; Minatoya *et al*. 2017b; Minatoya *et al*. 2018; Miodovnik *et al*. 2011; Perera *et al*. 2012; Roen *et al*. 2015), and most of these effects were sex-specific.

The actual mechanisms accounting for long-term effects of early-life exposure to BPA remain unclear. One hypothesis is that prenatal exposure to BPA might lead to health outcomes in the offspring through epigenetic alterations *in utero* because epigenetics is an *intrinsic* biological mechanism that can be affected by *extrinsic* environmental factors in humans.

DNA methylation is among the most studied mechanisms of epigenetic regulation (Mileva *et al*. 2014). It occurs by the addition of a methyl group to a cytosine mostly at cytosine-guanine dinucleotide (CpG) loci and acts like a gene expression switch (Hackett and Surani 2013). Evidence from experimental studies suggests that DNA methylation changes in the offspring can occur in response to developmental BPA exposure (Alonso-Magdalena *et al*. 2016; Ideta-Otsuka *et al*. 2017; Mileva *et al*. 2014; Singh and Li 2012a). Previous human cohort studies showed that prenatal BPA exposure associated with DNA methylation profiles of fetal liver genes (Faulk *et al*. 2016; Nahar *et al*. 2014; Nahar *et al*. 2015) and metabolism-related genes of the offspring (Goodrich *et al*. 2016; Montrose *et al*. 2018). Furthermore, it has been shown that BPA-induced epigenetic effects are usually sex-specific (McCabe *et al*. 2017; Montrose *et al*. 2018).

Genome-wide methylation analysis allows a hypothesis-free assessment of epigenetic alterations in relation to the environmental factors (Christensen and Marsit 2011); however, only one cohort study from Germany showed an association between maternal urinary BPA levels (low levels with <7.6 ng/mg creatinine; *n*=102 and high levels with >15.9 ng/mg creatinine; *n*=101) and genome-wide DNA methylation in cord blood samples regardless of infant's sex (Junge *et al*. 2018). We aimed to examine cord blood DNA methylation changes in association with BPA exposure by an epigenome-wide association study (EWAS) in a Japanese cohort. In addition, analyses were also performed to determine sex-specific differences in BPA exposure-associated methylation profiles.

B．研究方法

450K DNA methylation analysis. We assessed DNA methylation in cord blood samples from 277 mother-child pairs from the Sapporo cohort of the Hokkaido Study using the *Illumina HumanMethylation 450 BeadChip*. After quality control (Aryee *et al*. 2014), functional normalization (Fortin *et al*. 2014) was applied to the raw data, and normalized beta (β) values, ranging from 0-1 for 0% to 100% methylated, were obtained. We applied the ComBat method to adjust methylation data for sample plate to reduce a potential bias due to batch effects (Leek *et al*. 2012). Combat-transformed *M*-values (logit-transformed β-values) were back-transformed to *β-values* that were used for subsequent data analyses.

Exposure assessment. BPA levels were measured in cord blood by using isotope dilution liquid chromatography-tandem mass spectrometry (ID-LC/MS/MS) at IDEA Consultants, *Inc*. (Shizuoka, Japan) as described previously (Yamamoto *et al*. 2016). The LOQ of BPA was 0.04 ng/mL.

Data analysis. For the 87 samples below the LOQ (0.04 ng/mL), we assigned a value of half the detection limit (0.02 ng/mL). Cord blood cell proportion was estimated by the method implemented in the R/Bioconductor package *minfi* (Bakulski *et al*. 2016). Using *limma* package in R, robust linear regression analyses (Fox and Weisberg 2011) were applied to determine the associations of β-value at each CpG site with BPA natural log (*ln*)-transformed concentrations, adjusted for maternal age, educational levels, pre-pregnancy BMI, smoking during pregnancy, gestational age, infant sex, and cord blood cell estimates for $CD4^+$ T cells, $CD8^+$ T cells, granulocytes, monocytes, B cell and nucleated red blood cells. For multiple comparisons, *p*-values were adjusted by the false discovery rate (FDR) to obtain *q*-values. We further stratified the analysis by infant sex and compared CpGs with uncorrected *p*-value <0.0001 to confirm the sex-specific effect on DNA methylation changes as we had too few FDR-significant findings. Statistical analyses were performed using *minfi*, *sva*, and *limma* packages in R *ver*. 3.3.2 and Bioconductor *ver*. 3.3.

To further analyze underlying genetic networks of BPA-associated CpGs, we imported and analyzed genes related to DMPs surviving an FDR <0.05 using GeneMANIA (Warde-Farley *et al*. 2010) (https://genemania.org/) bioinformatics software with default parameters. We also assessed the differentially methylated CpGs with *p*-value <0.0001 for functional enrichment with GO terms and KEGG pathways (Kanehisa *et al*. 2002) via the gometh function in the *missMethyl* package in R/Bioconductor (Phipson *et al*. 2016).

Replication study. Eleven mother-infant pairs from a Taiwanese cohort had both BPA levels in maternal urine samples and *β-values* at 450K CpGs from cord blood DNA. Details of the study population have been published elsewhere (Huang *et al*. 2017). Linear regression analyses adjusted for child sex were applied to determine the associations of the β-value with *ln*-transformed BPA levels. Due to the very small sample size (n=11), we did not stratify the analysis by sex.

（倫理面への配慮）

Written informed consents were obtained from all participants. The institutional Ethical Board for human gene and genome studies at the Hokkaido University Graduate School of Medicine and the Hokkaido University Center for Environmental and Health Science approved the study protocol. The study protocol of Taiwan cohort was approved by the Institutional Review Board of Cathay General Hospital (CGH) in Taipei, Taiwan. All experiments were performed in accordance with relevant guidelines and regulations.

C．研究結果

Study characteristics. Maternal and infant characteristics and their relationship to BPA concentrations in cord blood are described in Table 1. BPA was detected in 68.6 % of cord blood samples. The median of cord blood BPA was 0.050 ng/mL (Interquartile range (IQR): < the limit of quantification (LOQ) – 0.075). The average ± standard deviation (s.d.) age of the mothers was 30.0 ± 4.9 years. Of the 277 newborns, 123 (44.4%) were male. None of the characteristics shown in Table 1 were significantly associated with BPA levels.

The median level of BPA, maternal characteristics, and gestational age were not significantly different between the infant sexes. The percentage of subjects with BPA values below the LOQ among the females (32.5%) was slightly higher than that among the males (30.1%).

Epigenome-wide association study. Volcano plots of EWAS analyses for all newborns, male infants, and female infants showed a different imbalance in positive versus negative methylation changes (Figure 1A), suggesting sex-specific global methylation shifts. We compared CpGs with uncorrected *p*-value < 0.0001 (45 CpGs in all newborns, 269 CpGs in male infants, and 291 CpGs in female infants) and observed a large portion of these were hypomethylated among all newborns (91%) and female infants (98%) (Figure 1B). In contrast, of the 269 CpGs among male infants, 236 CpGs (88%) became hypermethylated (Figure 1B).

Next, we studied differentially methylated probes (DMPs) with epigenome-wide significant methylation changes; a false discovery rate (FDR) *q*-value <0.05. Figure 2 shows Manhattan plots of genome-wide analyses of the association between cord blood DNA methylation and BPA exposure. The male- and female-specific DMPs are listed in Tables 2 and 3, respectively. In the male-only analysis, BPA levels in cord blood were associated with hypermethylation of 22 DMPs and hypomethylation of 6 DMPs. Among the female infants, BPA levels were associated with hypomethylation of 16 DMPs. There were no CpGs with FDR <0.05 in all newborns (Figure 2A); however, the directions of methylation changes at DMPs observed in all infants were consistent with those observed in the sex-stratified analyses (see Tables 2 and 3).

We explored whether FDR-significant DMPs identified in the Sapporo cohort also showed the same direction of methylation change in a Taiwan cohort (the right columns in Tables 2 and 3). Of the DMPs, one male- and two female-specific DMPs were not available for the analysis. Ten of the fourteen female-

specific DMPs showed the same direction of methylation changes (hypomethylation) in both cohorts, whereas the direction of methylation changes in the male-specific DMPs was not reproducible (9 of 27 male-specific DMPs).

Network analysis of DMPs. Genes annotated to DMPs (28 male-specific and sixteen female-specific genes) were analyzed by network analysis using GeneMANIA (Warde-Farley *et al*. 2010) (https://genemania.org/) that is based on known genetic and physical interactions, shared pathways and protein domains as well as protein co-expression data. Two male-specific genes (*LOC401242* and *LOC441455*) were not available for GeneMANIA analysis; therefore, only 26 of the 28 male-specific genes were included in this analysis. The analyses showed that all male-specific genes, except one (C6orf52), formed a compact cluster showing co-expression, genetic interaction, and colocalization (see Figure 3). Conversely, among the 16 female-specific genes, only six genes showed three disperse clusters of co-expression (Figure 4). The rest of the genes showed no interaction with others.

Gene ontology analysis. We also investigated the underlying biology that may be affected by BPA-associated variations in a sex-specific manner. As we had too few FDR-significant findings to perform an enrichment analysis on only those hit (28 DMPs in males and 16 DMPs in females), we tested for gene ontology (GO) terms and Kyoto Encyclopedia Genes and Genomes (KEGG) pathways (Kanehisa *et al*. 2002) enrichment among the CpGs associated with BPA levels with p <0.0001. Four GO terms among the female infants were significant at an FDR <0.05 as shown in Table 4. None of the GO terms among the males were significant at the FDR threshold. In contrast, the gene set for both sexes were enriched with genes from numerous KEGG pathways. The top ten enriched pathways ranked by the lowest p-value, excluding the pathways for diseases, are shown in Table 5. Among males, six of ten pathways are involved in signal transduction – MAPK signaling pathway, AMPK signaling pathway, Rap1 signaling pathway, Signaling pathways regulating pluripotency of stem cells, mTOR signaling pathway, and Phospholipase D signaling pathway. Among the ten pathways enriched among the female, three pathways were associated with the endocrine system – estrogen signaling pathway, relaxin signaling pathway, parathyroid hormone synthesis, secretion and action.

D．考察

Even though BPA levels in this study were relatively lower (Minatoya *et al*. 2017b) than those in the previous studies on cord blood BPA levels (Aris 2014; Chou *et al*. 2011; Kosarac *et al*. 2012), we found substantial sex differences in methylation changes associated with BPA exposure. Among males, BPA exposure was more frequently associated with hypermethylation than with hypomethylation, whereas it was associated predominantly with hypomethylation among female infants. Genes annotated to these CpGs also showed sex differences in the genetic network and functional enrichment analyses. Our results suggest that even at low levels, BPA exposure may impact DNA methylation status at birth in

a sex-specific manner.

Methylation changes derived from exposures to BPA showed notable sex-specific differences – the inversed direction of effects on methylation changes might be responsible for the lack of significant changes among all newborns. The observed preference of BPA-induced hypomethylation in females, as opposed to hypermethylation among males, is consistent with the findings from studies on blood-based methylation alterations that showed BPA-induced hypomethylation in women (Hanna *et al*. 2012) and in young girls (Kim *et al*. 2013).

While ten of the 14 female-specific DMPs (71%) had the same direction of methylation changes (hypomethylation) in both Sapporo and Taiwanese cohorts, only nine of the 27 male-specific DMPs showed the same direction of methylation changes in both cohorts (Tables 2 and 3). Effects of BPA on DNA methylation in the male infants has not been clarified (Martin *et al*. 2018). Further studies would be required to evaluate its effects among the males.

In addition to the opposed direction of effect between sexes, the network analyses of genes annotated to FDR-corrected DMPs showed the sex-difference (Figures 3 and 4). The male-specific genes clustered into a complex interconnected network, whereas the female-specific genes were isolated. In contrast, with regard to the gene pathways, the female-specific CpGs with *p*-value <0.0001 included genes that were significantly enriched for GO terms related to cell adhesion (FDR <0.05) – homophilic cell adhesion via plasma membrane adhesion molecules, cell-cell adhesion via plasma membrane adhesion molecules, cell-cell adhesion, and calcium ion binding (Table 4). On the other hand, genes annotated to the male-specific probes were not enriched in any GO terms with an FDR threshold. With respect to KEGG pathways, it should be noted that enrichment in the MAPK signaling pathway and the estrogen signaling pathway were observed among the genes annotated to male- and female-specific probes, respectively. According to Singh and Li (Singh and Li 2012b), mitogen-activated protein (MAPK) and estrogen receptor (ESR) were included in most frequently curated BPA-interacting genes/proteins.

Ｅ．結論

In conclusion, this epigenome-wide study suggested that even relatively low levels of exposure to BPA impact DNA methylation status at birth in a sex-specific manner. There may be a potential susceptibility difference in relation to BPA exposure between males and females. Further studies are needed to confirm our findings and to investigate their relevance to sex-specific adverse health outcomes.

Ｆ．研究発表

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G. 知的財産権の出願・登録状況

該当なし

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Table 1. Maternal and infant characteristics and their relationship with cord blood BPA levels (ng/mL).

		N (%) or Mean ± SD	Median (25th, 75th) or correlation (ρ)	p
BPA Concentration in cord blood (ng/ml)				
		0.056 ± 0.03	0.05 (0.020, 0.075)	
Maternal Charactristic				
Maternal Age (year)[a]		30.0 ± 4.9	ρ= -0.031	0.613
Prenatal-BMI (kg/m^2)[a]		20.9 ± 2.9	ρ= -0.031	0.605
Parity[b]	0	145 (52.3)	0.054 (0.020, 0.076)	0.407
	≧ 1	132 (47.7)	0.047 (0.020, 0.074)	
Educational level (year)[b]				
	≦ 12	123 (44.4)	0.050 (0.020, 0.076)	0.990
	> 12	154 (55.6)	0.052 (0.020, 0.070)	
Annual household income (million yen)[c]				
	< 3	51 (18.5)	0.055 (0.020, 0.072)	0.829
	3-5	144 (52.4)	0.053 (0.020, 0.076)	
	5-7	59 (21.5)	0.048 (0.020, 0.068)	
	> 7	21 (7.6)	0.044 (0.020, 0.085)	
Smoking during pregnancy[b]				
	No	234 (84.5)	0.051 (0.020, 0.075)	0.941
	Yes	43 (15.5)	0.056 (0.020, 0.072)	
Alcohol consumption during pregnancy[b]				
	No	183 (66.1)	0.052 (0.020, 0.075)	0.831
	Yes	94 (33.9)	0.047 (0.020, 0.075)	
Caffeine intake during pregnancy (mg/day)[a]				
		148.7 ± 121.9	ρ= -0.012	0.843
Infant Characteristic				
Geatation age (week)[a]		39.8 ± 1.0	ρ= -0.003	0.964
Sex[b]	Male	123 (44.4)	0.056 (0.020, 0.075)	0.429
	Female	154 (55.6)	0.047 (0.020, 0.073)	
Birth weight (g)[a]		3131.3 ± 333.5	ρ= 0.060	0.324

[a]Spearman's correlation test (ρ), [b]Mann-Whitney U-test, [c]Kruskal-Wallis test

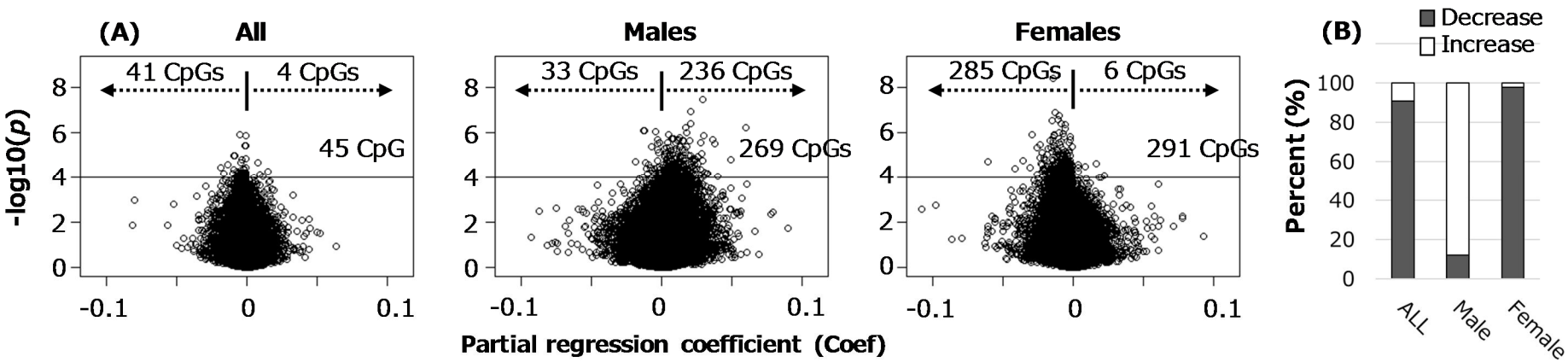

Figure 1. (A) Volcano plots of the $\log_{10}$(*p*-values) versus the magnitude of effect (Coef) for the genome-wide analysis of the association between BPA exposure and DNA methylation in cord blood among all newborns, male infants, and female infants. Horizontal lines represent a *p*-value <0.0001. (B) The percentage of decreased- and increased-CpGs with *p* <0.0001 found in the analyses for all, male, and female infants.

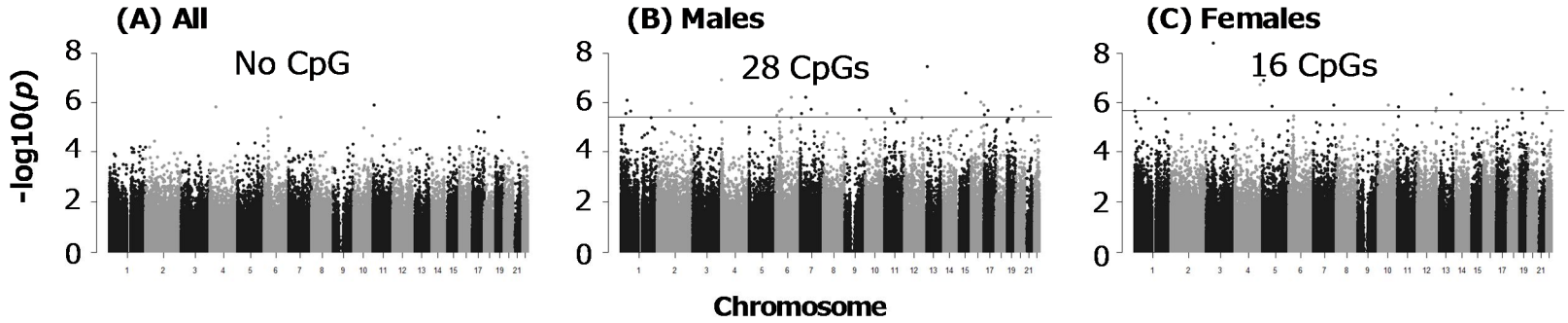

Figure 2. Manhattan plots of *p*-value associations between BPA exposure and DNA methylation across chromosomes in analyses for (A) all newborns, (B) male infants, and (C) female infants.
Horizontal lines represent the significance threshold of FDR q <0.05.

Table 2. 28 CpGs with DNA methylation levels in cord blood associated with BPA concentrations with FDR q < 0.05 in analysis for male infants

CpG	CHR	Gene	Relation to gens	Relation to island	Sapporo				Taiwan[a]		Replicated for the direction
					Male infants[b]		All newborns[c]		All newborns		
					Coef[d]	*p*-Value	Coef[d]	*p*-Value	Coef[d]	*p*-Value	
cg25275331	1	ZMPSTE24	1stExon	island	-0.013	7.91E-07	-0.003	0.100	0.015	0.239	
cg21115004	1	SLC35D1	TSS1500	shore	0.015	2.15E-06	0.002	0.241	0.011	0.614	✓
cg02920421	1	KPNA6	TSS1500	shore	0.010	2.70E-06	0.004	0.034	0.002	0.584	✓
cg04627863	2	HJURP	IGR	open sea	0.018	1.01E-06	0.009	0.001	0.001	0.930	✓
cg08527179	2	TMSB10	TSS1500	island	0.012	1.98E-06	0.004	0.005	-0.022	0.713	
cg23605991	4	PDE6B	TSS1500	shore	0.021	1.19E-07	0.005	0.085	0.003	0.644	✓
cg01119278	6	DDO	Body	island	0.060	5.83E-07	0.017	0.094	-0.009	0.567	
cg00050375	6	ABCC10	Body	open sea	0.009	1.78E-06	0.002	0.189	-0.003	0.253	
cg20620326	6	LOC401242	IGR	open sea	0.020	2.20E-06	0.003	0.487	-0.006	0.603	
cg02330394	6	C6orf52	TSS1500	island	-0.003	3.19E-06	-0.001	0.050	0.006	0.703	
cg22674202	7	GPR141	IGR	open sea	0.017	5.97E-07	0.005	0.079	-0.007	0.124	
cg17833862	7	CLIP2	Body	island	0.007	1.85E-06	0.003	0.032	-0.001	0.684	
cg00507727	7	ICA1	TSS200	island	-0.002	2.65E-06	-0.001	0.002	0.017	0.362	
cg14253670	8	NEFL	IGR	shelf	0.028	2.63E-06	0.010	0.008	-0.005	0.217	
cg13481969	9	LOC441455	IGR	island	-0.003	1.94E-06	-0.002	6.28E-05	0.038	0.542	
cg03470671	11	PRDM11	1stExon	open sea	0.012	1.71E-06	0.004	0.021	-0.004	0.168	
cg07637188	11	MADD	Body	open sea	0.011	2.19E-06	0.004	0.014	-0.000	0.872	
cg03687707	11	COX8A	IGR	shelf	0.004	2.68E-06	0.002	0.022	-0.002	0.072	
cg21372595	12	ATF7IP	IGR	shore	-0.012	8.53E-07	-0.005	0.013	0.000	0.898	
cg20981000	13	MTMR6	IGR	open sea	0.030	3.44E-08	0.004	0.313	0.001	0.949	✓
cg20796298	15	IGDCC4	Body	shore	0.021	3.97E-07	0.006	0.069	-0.000	0.997	
cg00120998	16	CENPT	TSS200	island	-0.005	9.42E-07	-0.002	0.023	0.020	0.406	
cg07130392	16	SPG7	Body	island	0.005	1.20E-06	0.001	0.187	0.000	0.658	✓
cg23798387	17	KIAA0100	TSS1500	shore	0.036	1.99E-06	0.008	0.203	0.014	0.595	✓
cg06126721	17	SLC43A2	3'UTR	island	0.038	3.01E-06	0.007	0.234	-0.009	0.394	
cg17922329	19	FXYD5	5'UTR	island	0.007	1.77E-06	0.002	0.077	0.043	0.148	✓
cg13636640	20	DNMT3B	TSS1500	shore	0.030	1.38E-06	0.011	0.010	NA		NA
cg19353578	22	PDGFB	Body	shelf	0.016	2.23E-06	0.003	0.141	0.002	0.769	✓

Abbreviations: Chr: chromosome, IGR: intergenic region, TSS: transcription start site, TSS200: 200 bases from TSS, TSS1500: 1500 bases from TSS, body: gene body: UTR: untranslated region, NA: not available.

[a] Linear regression analyses adjusted for child sex were applied to determine the associations of DNA methylation levels with *ln*-transformed BPA levels in Taiwan cohort.

[b]Adjusted for maternal age, maternal educational levels, maternal pre-pregnancy BMI, maternal smoking during pregnancy, gestational age, and cord blood cell estimates

[c]Adjusted for maternal age, maternal educational levels, maternal pre-pregnancy BMI, maternal smoking during pregnancy, gestational age, infant sex, and cord blood cell estimates.

[d]Partial regression coefficient indicates absolute DNA methylation change per *ln*-unit increase in BPA concentration.

Table 3. 16 CpGs with DNA methylation levels in cord blood associated with BPA concentrations with FDR q <0.05 among female infants.

CpG	Chr	Gene	Relation to gens	Relation to island	Sapporo				Taiwan[a]		Replicated for the direction
					Female infants[b]		All newborns[c]		All newborns		
					Coef[d]	p-Value	Coef[d]	p-Value	Coef[d]	p-Value	
cg12061021	1	RWDD3	TSS200	shore	-0.006	6.55E-07	-0.004	0.000	-0.006	0.784	✓
cg21461470	1	HIST2H2AA4	TSS1500	shore	-0.010	1.01E-06	-0.006	0.001	0.005	0.864	
cg22927302	3	SEMA3B	TSS1500	open sea	-0.015	4.00E-09	-0.005	0.014			NA
cg23603782	4	GALNTL6	Body	open sea	-0.010	1.81E-07	-0.006	0.000	-0.001	0.636	✓
cg27629673	5	ADCY2	Body	open sea	-0.013	1.27E-07	-0.005	0.029	-0.000	0.925	✓
cg22465281	5	NLN	TSS1500	shore	-0.014	1.36E-06	-0.005	0.036			NA
cg19734222	7	PRSS58	IGR	open sea	-0.030	1.25E-06	-0.011	0.030	0.003	0.710	
cg11820931	10	DDX21	Body	shore	-0.013	1.23E-06	-0.009	1.00E-05	-0.006	0.348	✓
cg08710564	11	ST5	5'UTR	open sea	-0.007	1.45E-06	-0.005	1.24E-06	-0.001	0.366	✓
cg17339927	12	FBRSL1	IGR	shore	-0.014	1.63E-06	-0.007	0.006	-0.005	0.245	✓
cg15193473	13	BIVM	5'UTR	island	-0.006	4.53E-07	-0.003	0.000	0.030	0.388	
cg27624753	16	CRAMP1L	Body	shelf	-0.007	1.10E-06	-0.005	6.65E-05	-0.005	0.092	✓
cg27638035	18	BRUNOL4	Body	open sea	-0.011	2.68E-07	-0.004	0.049	-0.003	0.333	✓
cg03636183	19	F2RL3	Body	shore	-0.016	2.81E-07	-0.005	0.067	-0.005	0.375	✓
cg07964219	21	COL18A1	Body	shore	-0.009	3.90E-07	-0.005	0.008	0.000	0.983	
cg15477600	22	SDF2L1	TSS1500	island	-0.002	1.57E-06	-0.001	0.004	-0.009	0.726	✓

Abbreviations: Chr: chromosome, IGR: intergenic region, TSS: transcription start site, TSS200: 200 bases from TSS, TSS1500: 1500 bases from TSS, body: gene body, UTR: untranslated region, NA: not available.

[a] Linear regression analyses adjusted for child sex were applied to determine the associations of DNA methylation levels with *ln*-transformed BPA levels in Taiwan cohort.

[b]Adjusted for maternal age, maternal educational levels, maternal pre-pregnancy BMI, maternal smoking during pregnancy, gestational age, and cord blood cell estimates

[c]Adjusted for maternal age, maternal educational levels, maternal pre-pregnancy BMI, maternal smoking during pregnancy, gestational age, infant sex, and cord blood cell estimates.

[d]Partial regression coefficient indicates absolute DNA methylation change per *ln*-unit increase in BPA concentration.

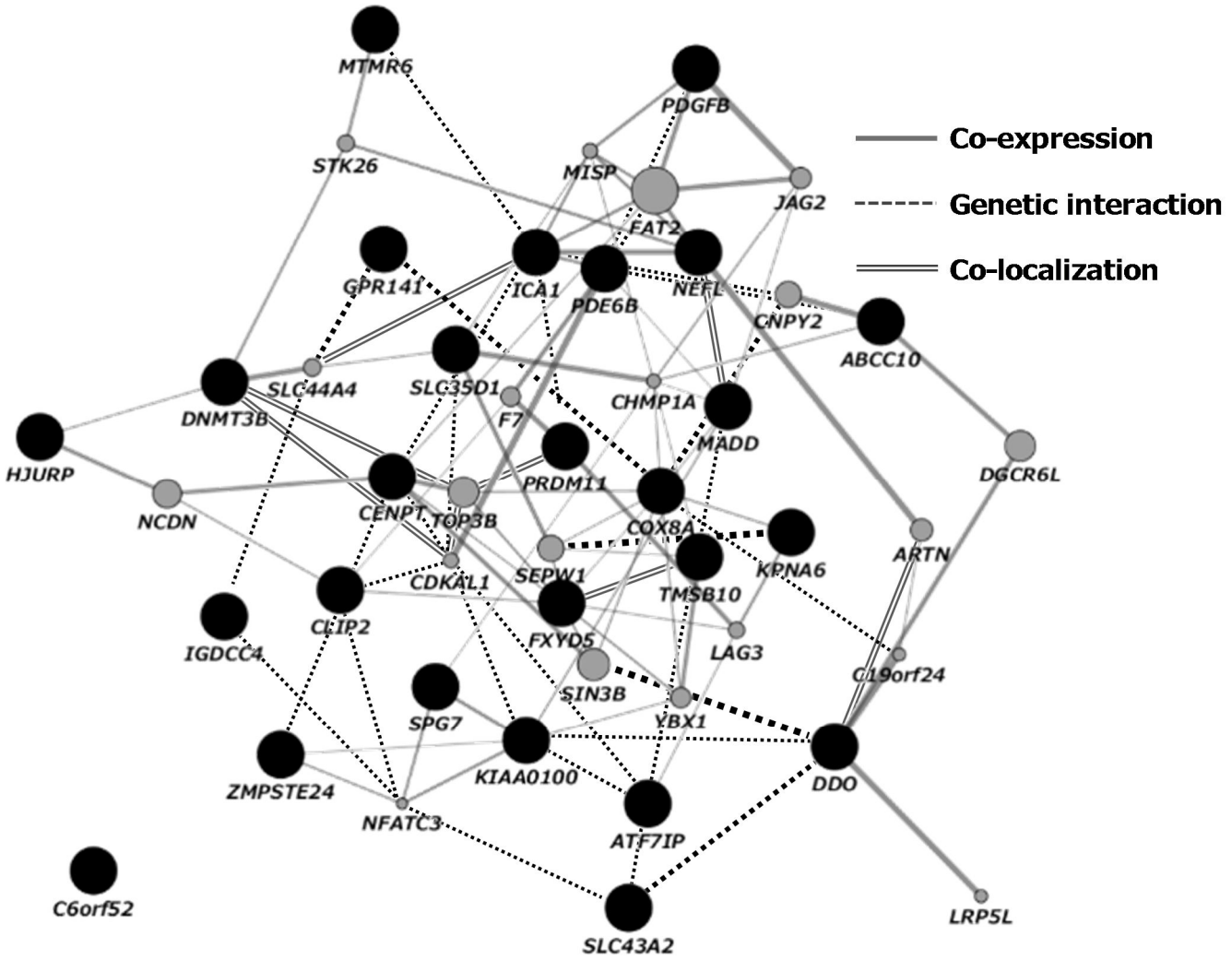

Figure 3. Gene network analysis using GeneMANIA. Dark circles represent genes associated with the 26 DMPs found to be related to BPA exposure in the male-only analysis. Light circles represent additional genes predicted by GeneMANIA based on genetic and physical interactions.

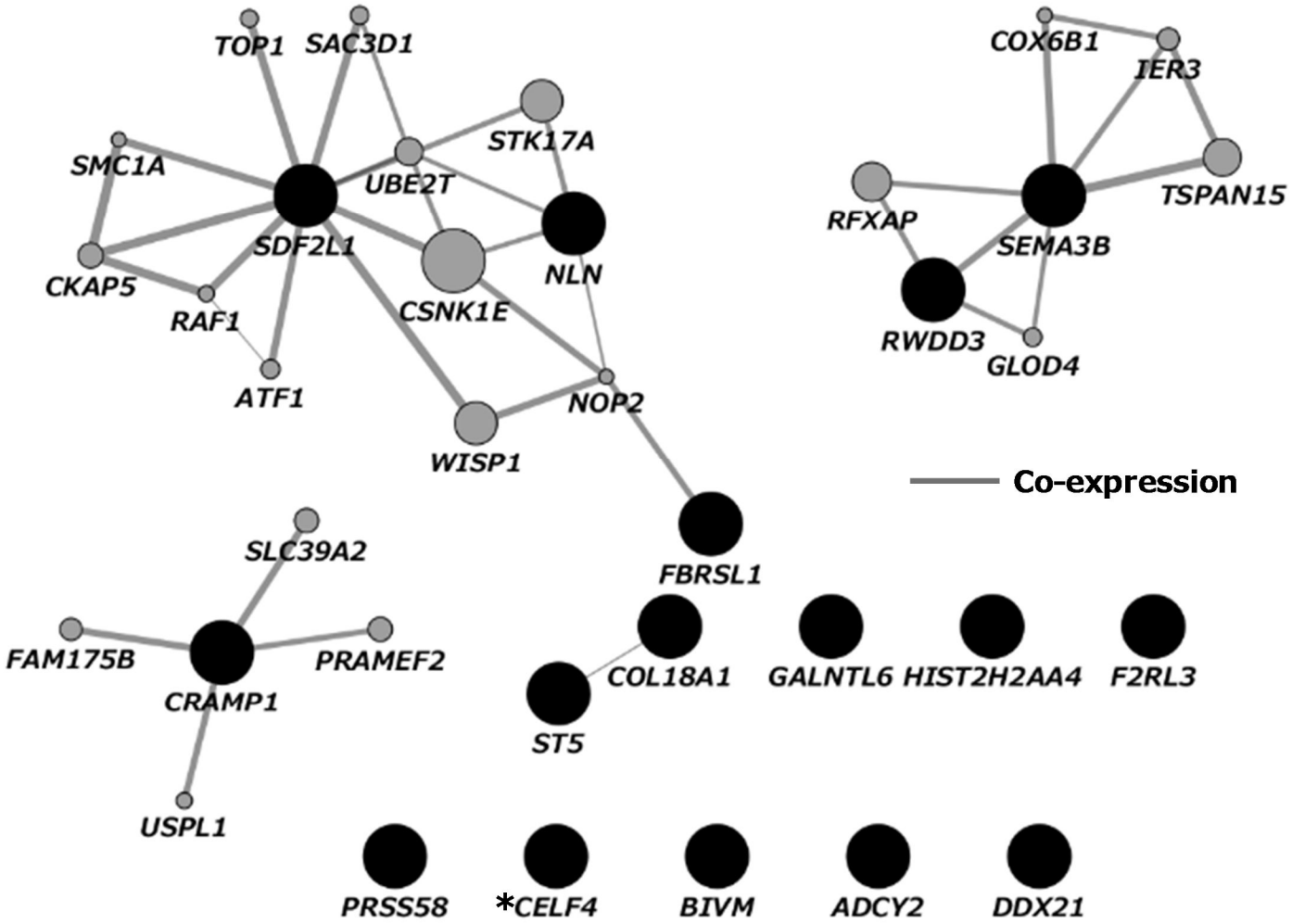

Figure 4. Gene network analysis using GeneMANIA. Dark circles represent genes associated with the 16 DMPs found to be related to BPA exposure in the female-only analysis. Light circles represent additional genes predicted by GeneMANIA based on genetic and physical interactions. **CELF4*, an alias for *BRUNOL4*

Table 4. Significantly enriched GO terms (FDR <0.05) for the CpGs with *p*-value <0.0001 from the female-only analysis.

GO term	Ontology	N	DE	p-Value	FDR
homophilic cell adhesion via plasma membrane adhesion molecules	BP	149	22	4.57E-12	9.46E-08
cell-cell adhesion via plasma-membrane adhesion molecules	BP	210	22	5.28E-10	5.46E-06
cell-cell adhesion	BP	845	38	1.92E-07	0.001
calcium ion binding	MF	652	30	2.17E-06	0.011

N: number of genes represented by a GO term, DE: number of genes from the GO term that were included in the CpGs with p <0.0001, FDR: FDR-adjusted *p*-value for enrichment, BP: biological process, MF: molecular function

Table 5. Top 10 enriched KEGG pathways ranked by the lowest *p*-value associated with genes annotated to the CpGs with $p<0.0001$ from the male- and female-only analyses.

KEGG pathway description	N	DE	P.DE	FDR
Male only analysis				
Phagosome	142	5	3.85E-05	0.002
MAPK signaling pathway	283	7	6.28E-05	0.002
AMPK signaling pathway	117	5	7.51E-05	0.002
Focal adhesion	191	6	8.16E-05	0.002
Rap1 signaling pathway	203	6	8.60E-05	0.002
Signaling pathways regulating pluripotency of stem cells	135	5	1.35E-04	0.003
mTOR signaling pathway	147	5	1.59E-04	0.003
Cellular senescence	153	5	1.72E-04	0.003
Phospholipase D signaling pathway	142	5	2.07E-04	0.003
Natural killer cell mediated cytotoxicity	105	4	2.29E-04	0.003
Female only analysis				
cAMP signaling pathway	192	8	9.13E-07	1.5E-04
Estrogen signaling pathway	136	7	9.36E-07	1.5E-04
Relaxin signaling pathway	125	6	1.19E-05	0.001
Th17 cell differentiation	102	5	3.43E-05	0.001
Wnt signaling pathway	140	6	3.54E-05	0.001
Cell cycle	121	5	7.76E-05	0.002
Platelet activation	122	5	9.80E-05	0.002
B cell receptor signaling pathway	68	4	1.20E-04	0.003
Parathyroid hormone synthesis, secretion and action	105	5	1.21E-04	0.003
Cytokine-cytokine receptor interaction	265	5	2.64E-04	0.004

N: total genes in the KEGG pathway, DE: the number of genes within the CpGs with $p<0.0001$, FDR: FDR-adjusted *p*-value for enrichment