

SUPPLEMENTARY MATERIALS

Methods

Placental Sample Preparation and PAHs Assessment The procedures have been described in our previous study^[1]. Placentas were thawed at 4 °C before being processed, and then about 10 g wet placental tissue was taken within 2 cm on the fetal side around the point of cord attachment. Each sample was spiked with recovery surrogate standards, homogenized in hexane/acetone solvent mixture (1:1 by volume), and extracted three times by ultrasonication and vortex mixing. Lipophilic material in the extractant was determined gravimetrically and then removed by gel permeation chromatography, followed by silica gel column chromatography. After concentration, samples were spiked with internal standards and analyzed with an Agilent 7890A-5975C gas chromatograph and mass spectrometer in electron impact ionization mode. Black samples were added with each batch of seven placental samples. The personnel involved in the experiment were not aware of the case-control information.

Placental DNA-PAHs Assessment As described previously^[2], DNA was extracted from about 1 g wet placenta tissue which was on the fetal side. Sampled tissue was thawed, minced, and washed manually with ice-cold PBS (pH 7.0) or saline to remove blood. The minced placental tissue was washed again followed by centrifugation. The tissue pellet was homogenized in an electric blender with a solution of PBS and centrifuged again. DNA was extracted by treating this placental tissue pellet with a mixture of 2 mL digestion buffer [25 mmol/L EDTA, 10 mmol/L Tris-Cl (pH = 7.4), 10 mmol/L NaCl, 1% SDS, 200 µg/mL proteinase K, and 20 µg/mL RNase A], overnight at 37 °C, followed by deproteinization with phenol/chloroform/isoamyl alcohol (25:24:1). DNA was precipitated with ethanol/NaCl, spooled out, washed with 70% ethanol, and air-dried. DNA concentration and purity were measured spectrophotometrically by absorbance at 260 and 280 nm. The A260/A280 ratio for all samples was between 1.8 and 2.0.

PAH-DNA adducts were determined by the nuclease P1-enhanced ³²P-postlabeling method. Briefly, aliquots of 5 µg DNA samples were dried over Speedvac and digested by a mixture of micrococcal endonuclease (MN) and spleen phosphodiesterase (SPD) at 37 °C overnight. Nuclease P1 was used for adduct enrichment, followed by incubating the mixture with [γ -³²P]ATP and T4 polynucleotide kinase. The labeled DNA adducts were separated by multidirectional anion-exchange thin layer chromatography (TLC) on 10 cm × 10 cm PEI-cellulose plates. Solvent systems used for TLC were the following: D1: 2.3M sodium phosphate, pH 5.7; D2: 4.05M lithium formate + 7.65M urea, pH 3.5; D3: 0.72M sodium phosphate, 0.42M tris, 7.65M urea, pH 8.2. The radioactivity was determined by phosphoimager (Typhoon Trio, GE Healthcare) after exposing to a storage phosphor screen (35 cm × 43 cm) at room temperature for 2 h. DNA adduct levels were quantified and expressed as adducts per 10⁸ nucleotides.

Placental metal(loid)s Assessment The procedures also have been described in our previous study^[3]. After being thawed at 4 °C, a ring-shaped placental sample within 3 cm around the umbilical insertion point was excised. The placental samples were cleaned three times with ultrapure water to remove residual blood. Then four pieces of tissue at each quarter of the circular placental tissue, totally weighing about 6.0 g, were excised with titanium alloy tissue scissors. The cleaned placental samples were kept at -80 °C for 8 h before freeze drying with a lyophilizer (ALPHA2-4 LD plus, Christ, Germany) in a 24 h process to remove excessive water. Approximately 0.1 g freeze-dried placental sample was digested in an 8.0 mL quartz tank with 0.6 mL nitric acid (UP-grade) and 0.2 mL ultrapure purity water, for 2 h, and the volume of digested solution was increased to 8.0 mL and mixed well. K, Ca, Na, Mg, Zn, and Fe were diluted 10 times before their amounts were determined using an inductively coupled plasma-emission spectrometer (ICP-OES, iCap6000, Thermo Fisher Scientific, USA) with power: 1,150 KW; cooling gas: 15 L/min, auxiliary gas: 0.5 L/min; carrier gas 0.2 mpa; vertical observation height: 12 mm. An aliquot of 1.5 mL digested sample was mixed with 0.1 mL mixed internal standard (Indium, Rhodium) and 0.4 mL of high pure water in a 2.0 mL Eppendorf tube. The mixture was used to determine concentrations of Cr, Mn, As, and Se on an inductively coupled plasma mass spectrometer (7700x, Agilent, Santa Clara, CA, USA) with carrier gas flow: 1.0 L/min, helium gas flow: 4.5 mL/min, radio frequency power: 1,550 W, integral time: 300 ms, scanning mode: 3 points. Another 1.5 mL digested sample was prepared as before to detect the other metal(loid)s on an inductively coupled plasma mass spectrometer (Elan DRC II, PerkinElmer, Waltham, MA, USA) with atomized gas flow rate: 0.96 L/min, auxiliary gas flow rate: 1.87 L/min, plasma gas flow rate: 17.0 L/min, radio frequency power: 1,100 W, scanning mode: single point peak, resolution: 0.7–0.9 amu.

Supplementary Table S1. Target compounds and their abbreviations used in this study

Compound	Abbreviation
PAHs	
Fluorene	FLU
Phenanthrene	PHE
Anthracene	ANT
Fluoranthene	FLT
Pyrene	PYR
Benzo[a]anthracene	BaA
Chrysene	CHR
Benzo[b]fluoranthene	BbF
Benzo[k]fluoranthene	BkF
Benzo[g,h,i]perylene	BPE
Metal(loid)s	
Calcium	Ca
Ferrum	Fe
Kalium	K
Magnesium	Mg
Sodium	Na
Zinc	Zn
Chromium	Cr
Manganese	Mn
Arsenic	As
Selenium	Se
Boron	B
Aluminum	Al
Titanium	Ti
Germanium	Ge
Strontium	Sr
Lithium	Li
Cobalt	Co
Nickel	Ni
Molybdenum	Mo
Argentum	Ag
Cadmium	Cd
Stannum	Sn
Stibium	Sb
Barium	Ba
Cesium	Cs
Uranium	U
Cuprum	Cu
Rubidium	Rb
Mercury	Hg
Plumbum	Pb
Lanthanum	La
Cerium	Ce
Praseodymium	Pr

Continued

Compound	Abbreviation
Neodymium	Nd
Samarium	Sm
Gadolinium	Gd
Dysprosium	Dy
Yttrium	Y

Supplementary Table S2. Basic characteristics of the pregnant women with neural tube defects (cases) and healthy (controls) infants in case-control groups^a

Characteristics	Controls (n = 50)	Cases (n = 80)	P ^b
Maternal age (years)			0.340
< 25	15 (32)	31 (39)	
25–29	11 (23)	23 (29)	
≥ 30	21 (45)	25 (32)	
Maternal education			0.225
Primary or lower	5 (10)	15 (19)	
Junior high school	41 (82)	54 (68)	
High school or above	4 (8)	10 (13)	
Maternal occupation			0.163
Farmer	45 (92)	64 (83)	
Nonfarmer	4 (8)	13 (17)	
Parity			0.124
1	23 (46)	45 (60)	
≥ 2	27 (54)	30 (40)	
Previous birth defects history			0.081 ^c
Yes	0 (0)	6 (8)	
No	50 (100)	73 (92)	
Conception season			0.391
Spring	13 (27)	19 (25)	
Summer	5 (10)	15 (20)	
Autumn	6 (13)	13 (17)	
Winter	24 (50)	29 (38)	
Periconceptional folate supplementation			0.501
Yes	6 (13)	7 (9)	
No	41 (87)	71 (91)	
Fever or flu during early pregnancy			0.001
Yes	4 (8)	25 (33)	
No	46 (92)	50 (67)	
Maternal passive smoking			0.087
Yes	24 (48)	50 (63)	
No	26 (52)	29 (37)	

Note. ^a[n (%)], numbers shown in this table were after before imputation; ^bPearson chi-square test; ^cFisher's exact test.

Supplementary Table S3. Limit of detection and detection rate of polycyclic aromatic hydrocarbons (PAHs) and metal(loid)s in placental tissue

Compounds	Limit of detection (LOD)	Detection rate (%)
PAHs (ng/g lipid)		
FLU	0.07	100
PHE	0.07	100
ANT	0.07	100
FLT	0.14	100
PYR	0.14	100
BaA	1.02	85
CHR	0.14	100
BbF	0.07	96
BkF	0.07	97
BPE	0.07	86
Σ_{10} PAHs		-
PAH-DNA adducts (per 10^8 nucleotides)		100
Metal(loid)s (ng/g dry weight)		
Ca	0.40	100
Fe	0.72	100
K	1.60	100
Mg	1.60	100
Na	1.57	100
Zn	0.05	100
Cr	0.21	100
Mn	0.85	100
As	5.33	98
Se	5.33	100
B	0.53	100
Al	10.67	100
Ti	1.07	100
Ge	0.96	100
Sr	1.07	100
Li	2.88	96
Co	0.11	100
Ni	0.53	100
Mo	0.21	100
Ag	0.32	95
Cd	0.32	100
Sn	0.75	100
Sb	0.32	98
Ba	0.21	100

Continued

Compounds	Limit of detection (LOD)	Detection rate (%)
Cs	0.11	100
U	0.02	100
Cu	3.20	100
Rb	0.42	100
Hg	0.75	100
Pb	1.28	100
La	0.06	100
Ce	0.04	100
Pr	0.01	100
Nd	0.03	100
Sm	0.04	100
Gd	0.21	86
Dy	0.11	92
Y	0.11	100

Supplementary Table S4. Association between placental single exposure and neural tube defects (NTDs) in a single-exposure exposure-wide association study (ExWAS) model

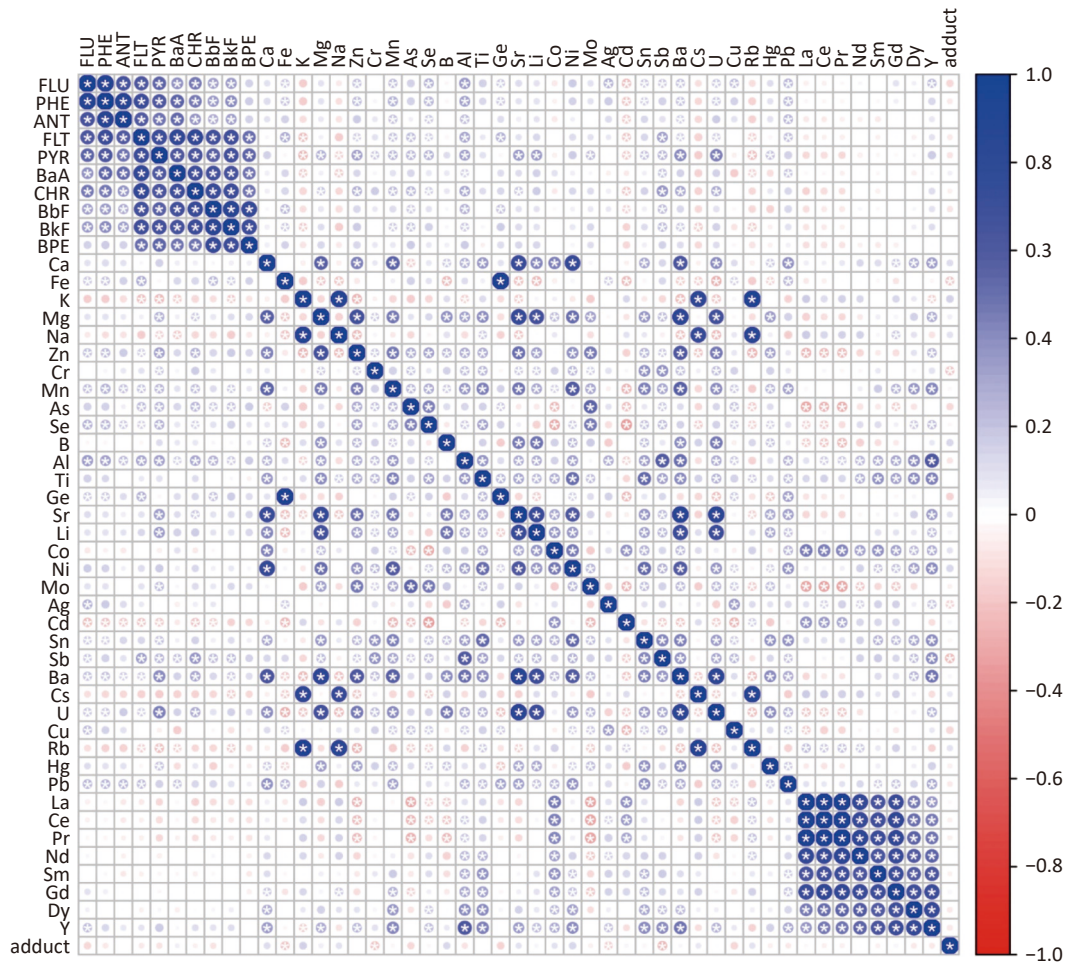
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Compounds	ORs (95% CIs)	<i>p</i>	<i>q</i>
Mo	3.77 (2.27–6.25)	< 0.001	< 0.001
Cd	0.31 (0.19–0.49)	< 0.001	< 0.001
Se	3.22 (1.99–5.21)	< 0.001	< 0.001
Zn	3.05 (1.90–4.92)	< 0.001	< 0.001
Mn	2.54 (1.66–3.89)	< 0.001	< 0.001
PYR	2.49 (1.62–3.81)	< 0.001	< 0.001
Σ ₁₀ PAHs	2.41 (1.58–3.68)	< 0.001	< 0.001
U	2.59 (1.63–4.11)	< 0.001	< 0.001
As	2.25 (1.49–3.42)	< 0.001	0.001
Rb	0.47 (0.31–0.70)	< 0.001	0.001
Ba	2.21 (1.46–3.36)	< 0.001	0.001
Co	0.44 (0.28–0.68)	< 0.001	0.001
FLU	2.08 (1.39–3.11)	< 0.001	0.001
K	0.48 (0.32–0.72)	< 0.001	0.001
Mg	2.15 (1.41–3.29)	< 0.001	0.001
PHE	2.04 (1.37–3.06)	0.001	0.002
CHR	1.93 (1.30–2.89)	0.001	0.004
Sb	1.87 (1.25–2.78)	0.002	0.006
FLT	1.84 (1.24–2.71)	0.002	0.006
Y	1.84 (1.24–2.75)	0.003	0.007
Na	0.56 (0.38–0.82)	0.003	0.007
Cs	0.56 (0.38–0.82)	0.003	0.007
ANT	1.75 (1.20–2.56)	0.004	0.008
Pb	1.79 (1.21–2.64)	0.004	0.008
Cu	1.77 (1.20–2.61)	0.004	0.008
Hg	1.81 (1.21–2.72)	0.004	0.008
Ni	1.71 (1.17–2.51)	0.006	0.011
Al	1.73 (1.17–2.55)	0.006	0.010
Sr	1.67 (1.15–2.44)	0.007	0.013
La	0.59 (0.40–0.87)	0.007	0.012
Sn	1.65 (1.14–2.40)	0.008	0.013
BkF	1.71 (1.15–2.54)	0.009	0.013
Pr	0.62 (0.42–0.90)	0.012	0.018
BbF	1.57 (1.06–2.30)	0.023	0.033
Ce	0.66 (0.46–0.96)	0.031	0.044
Cr	1.51 (1.04–2.20)	0.031	0.043
Ca	1.48 (1.03–2.12)	0.036	0.049
PAH-DNA adducts	0.69 (0.48–0.99)	0.043	0.057

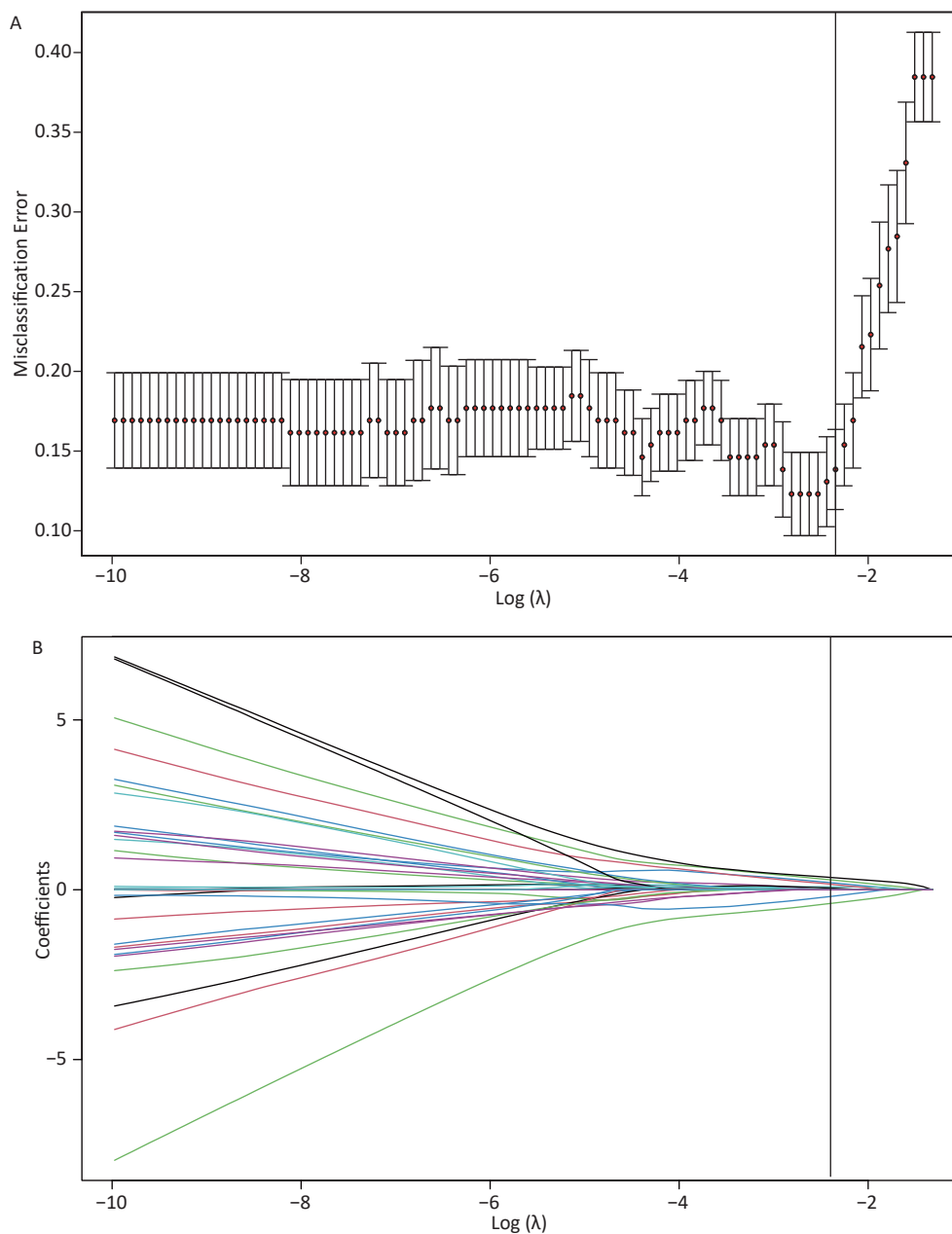
Compounds	ORs (95% CIs)	<i>p</i>	<i>q</i>
Ti	1.43 (1.00–2.05)	0.052	0.066
BaA	1.38 (0.95–1.99)	0.087	0.108
Sm	0.80 (0.55–1.14)	0.219	0.267
Nd	0.81 (0.57–1.15)	0.234	0.278
BPE	1.20 (0.84–1.73)	0.318	0.369
Ge	1.19 (0.82–1.72)	0.360	0.409
Li	1.17 (0.81–1.69)	0.411	0.457
Dy	0.86 (0.60–1.24)	0.413	0.449
Gd	0.87 (0.61–1.25)	0.450	0.479
Ag	0.90 (0.63–1.29)	0.561	0.584
B	1.07 (0.73–1.56)	0.741	0.756
Fe	0.97 (0.67–1.41)	0.887	0.887

Supplementary Table S5. Effect modification of metals on PAHs-NTDs associations, and PAHs on metals-NTDs associations

Independent variables	Effect modifiers	OR (95% CI)	P	P _{ME}
Metals on PAHs-NTDs associations				
PYR	Se_low	10.49 (2.14–51.37)	0.004	0.790
	Se_high	70.32 (2.25–2201.58)	0.015	
	Mo_low	15.00 (3.59–62.68)	< 0.001	
	Mo_high	17.48 (1.15–266.20)	0.039	
	Cd_low	10.47 (1.31–83.66)	0.027	
	Cd_high	20.05 (4.14–97.03)	< 0.001	
	U_low	7.04 (1.71–28.94)	0.007	
	U_high	40.92 (2.69–622.43)	0.008	
	Rb_low	5.89 (1.21–28.78)	0.028	
	Rb_high	13.00 (3.15–53.66)	< 0.001	
PAHs on metals-NTDs associations				
Se	PYR_low	12.84 (2.53–65.12)	0.002	0.790
	PYR_high	24.17 (2.27–256.87)	0.008	
Mo	PYR_low	14.91 (3.44–64.61)	< 0.001	0.968
	PYR_high	51.83 (2.68–1000.84)	0.009	
Cd	PYR_low	0.05 (0.01–0.25)	< 0.001	0.205
	PYR_high	0.15 (0.02–0.94)	0.043	
U	PYR_low	3.01 (0.75–12.09)	0.121	0.752
	PYR_high	3.98 (0.53–30.09)	0.181	
Rb	PYR_low	0.16 (0.04–0.61)	0.007	0.532
	PYR_high	0.46 (0.08–2.63)	0.381	



Supplementary Figure S1. Correlation heatmap between concentrations of target compounds measured in placenta. The blue circles represent positive correlations between compounds, while the red ones represent negative correlations; *indicates a significant correlation by Pearson correlation test, $P < 0.05$.



Supplementary Figure S2. Misclassification error of the LASSO regression model in function of the penalty parameter ($\log_{10} \lambda$) (the figures above) and the LASSO solution path with the coefficient profiles for polycyclic aromatic hydrocarbons (PAHs) and metals as a function of the penalty parameter ($\log_{10} \lambda$) (the figures below).

REFERENCES

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