

Supplementary Materials

Sex-specific effects of prenatal metal exposure on child anogenital distance: an advanced computational analysis

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Supplementary Table 1. Limits of detection for 10 metals in maternal plasma

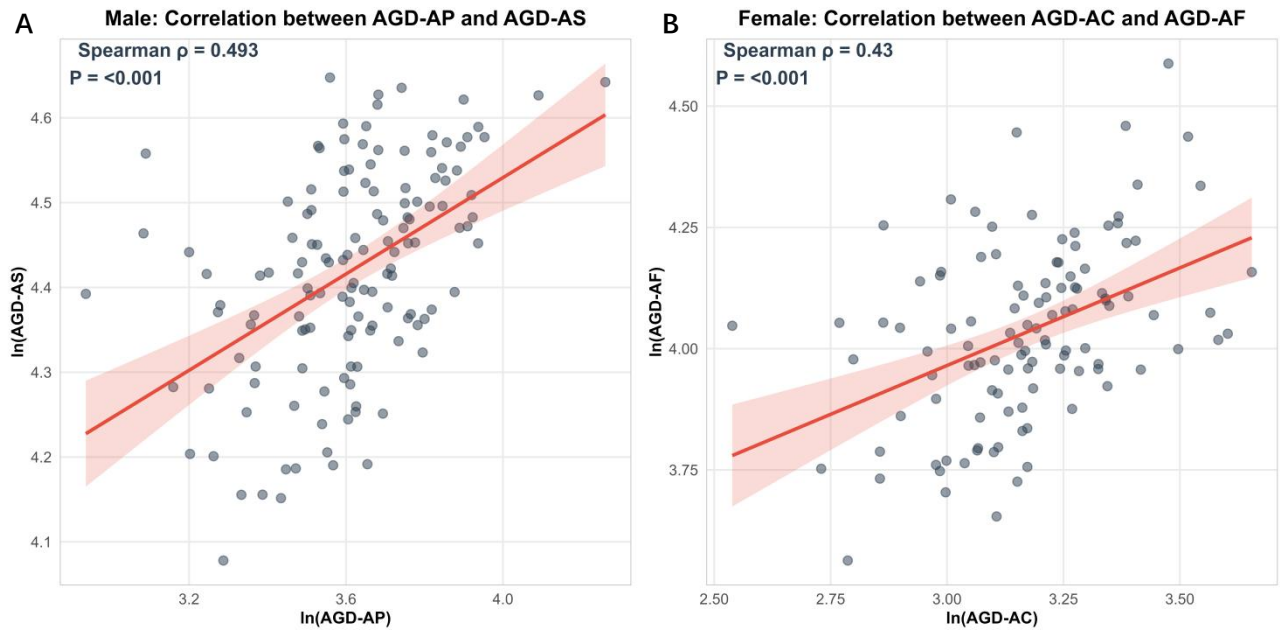
Metals	LOD (µg/L)
Cr	0.073
Ni	0.147
Cu	0.579
Zn	7.381
As	0.127
Cd	0.033
Sn	0.060
Sb	0.044
Hg	0.034
Tl	0.003
Pb	0.114

Notes: LOD, limit of detection. All metal concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7900 Series, Agilent Technologies, USA).

Supplementary Table 2. Sex-specific distribution of AGD measures in 2-year-old children

Variable	N	2.5th	25th	50th	75th	97.5th
AGD-AS	136	23.9	33.1	37.2	42.5	51.3
AGD-AF	112	16.2	21.3	23.9	26.6	35.5
AGD-AP	136	64.5	77.4	83.6	91.2	102.2
AGD-AC	112	41.3	51.4	56.7	62.9	84.7

Notes: Values are in millimeters (mm). Data are presented as selected percentiles of the distribution (2.5th, 25th, 50th, 75th, and 97.5th). Abbreviations: AGD-AS: anoscrotal distance in males; AGD-AF: anofourchette distance in females. AGD-AP: anopenile distance in males; AGD-AC: anoclitoral distance in females.

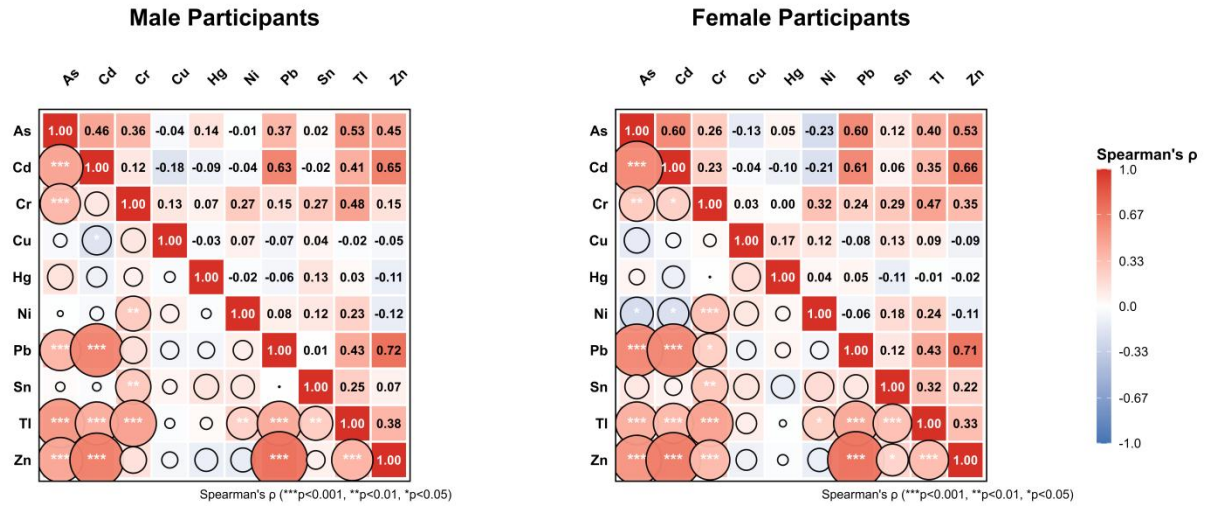


Supplementary Figure 1. Correlations between AGD measurements (natural log-transformed). Scatter plots show (A) the correlation between $\ln(\text{AGD-AP})$ and $\ln(\text{AGD-AS})$ in males ($n = 136$), and (B) the correlation between $\ln(\text{AGD-AC})$ and $\ln(\text{AGD-AF})$ in females ($n = 112$). Spearman correlation coefficients (ρ) and P values are shown in the upper-left corner. Red lines are linear regression fits with 95% confidence intervals (gray bands). Abbreviations: AGD-AP, anus-penis distance; AGD-AS, anus-scrotum distance; AGD-AC, anus-clitoris distance; AGD-AF, anus-fourchette distance; $\ln$, natural logarithm.

Supplementary Table 3. Distribution of first-trimester maternal plasma metal concentrations by infant sex

Metal (µg/L)	Sex	N	Detection rate						P value
			(%)	2.5th	25th	50th	75th	97.5th	
Cr	Male	136	100.00	0.443	0.586	0.740	0.903	1.817	0.604
	Female	112	100.00	0.437	0.608	0.718	0.978	1.625	
Ni	Male	136	100.00	0.937	1.270	1.771	2.348	3.470	0.210
	Female	112	100.00	0.850	1.251	1.639	2.075	3.098	
Cu	Male	136	100.00	1130.6	1452.8	1679.0	1879.6	2357.1	0.862
	Female	112	100.00	1150.1	1472.3	1680.7	1851.1	2348.7	
Zn	Male	136	100.00	535.5	717.0	802.7	1522.4	4995.5	0.055
	Female	112	100.00	553.6	755.0	912.8	1794.3	3993.3	
As	Male	136	100.00	0.288	0.500	0.709	1.179	13.666	0.053
	Female	112	100.00	0.294	0.571	0.864	1.893	13.905	
Cd	Male	136	61.76	0.017	0.017	0.056	0.119	0.293	0.714
	Female	112	69.64	0.017	0.017	0.062	0.121	0.341	
Sn	Male	136	78.68	0.030	0.063	0.085	0.125	0.250	0.196
	Female	112	65.18	0.030	0.030	0.083	0.116	0.541	
Hg	Male	136	100.00	0.073	0.138	0.183	0.257	0.463	0.446
	Female	112	100.00	0.075	0.146	0.190	0.263	0.475	
Tl	Male	136	100.00	0.021	0.089	0.113	0.138	0.252	0.582
	Female	112	100.00	0.040	0.086	0.111	0.134	0.252	
Pb	Male	136	100.00	0.662	1.836	3.621	6.382	18.303	0.339
	Female	112	100.00	1.042	2.025	4.077	6.816	15.462	

Notes: Values are percentiles (2.5th, 25th, 50th, 75th, and 97.5th) of the metal concentration distributions. *P* values were calculated using Wilcoxon rank-sum tests comparing males versus females. Metal concentrations are in µg/L. N indicates number of participants with complete data for each sex. Abbreviations: µg/L, micrograms per liter.



Supplementary Figure 2. Spearman correlation matrices of metal concentrations in the overall population and sex-stratified subgroups. Sample sizes: males $n = 136$; females $n = 112$. Heatmaps display Spearman's rank correlation coefficients (ρ) between concentrations of ten metals (Cr, Ni, Cu, Zn, As, Cd, Sn, Hg, Tl, Pb) in blood. Correlation coefficients are shown in the upper triangle. The lower triangle indicates statistical significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. Analyses were performed for the male participants and the female participants.

Supplementary Table 4. Sex-stratified associations between first-trimester plasma metal concentrations and AGD (AGD-AS/AGD-AF) in 2-year-old children

Metal	Model	AGD-AS (male)			AGD-AF (female)		
		Percent change (% 95% CI)	<i>P</i> value	<i>P</i> _{FD} <i>R</i>	Percent change (% 95% CI)	<i>P</i> value	<i>P</i> _{FD} <i>R</i>
As	Model 1	-0.93 (-4.27, 2.52)	0.588	0.77 9	-0.90 (-4.22, 2.54)	0.601	0.98 5
	Model 2	-0.84 (-4.16, 2.59)	0.623	0.77 9	-0.53 (-3.87, 2.93)	0.759	0.98 5
	Model 3	-1.23 (-4.58, 2.24)	0.479	0.77 9	-0.67 (-4.19, 2.98)	0.713	0.98 5
Cd	Model 1	-0.03 (-7.10, 7.59)	0.994	0.99 4	-1.11 (-8.04, 6.34)	0.762	0.98 5
	Model 2	-0.18 (-7.21, 7.37)	0.960	0.99 4	0.09 (-7.00, 7.71)	0.982	0.99 8
	Model 3	-0.31 (-7.43, 7.36)	0.935	0.99 4	-0.01 (-7.32, 7.89)	0.998	0.99 8
Cr	Model 1	-2.18 (-6.54, 2.39)	0.342	0.77 9	-0.59 (-4.79, 3.81)	0.788	0.98 5
	Model 2	-2.06 (-6.40, 2.49)	0.366	0.77 9	-0.11 (-4.34, 4.30)	0.959	0.99 8
	Model 3	-1.38 (-5.90, 3.36)	0.559	0.77 9	-0.73 (-5.00, 3.73)	0.742	0.98 5
Cu	Model 1	3.07 (-1.38, 7.72)	0.178	0.77 9	-1.46 (-6.43, 3.78)	0.575	0.98 5
	Model 2	2.57 (-1.89, 7.23)	0.260	0.77 9	-1.18 (-6.14, 4.04)	0.648	0.98 5
	Model 3	3.34 (-1.39, 8.30)	0.168	0.77 9	-1.65 (-6.65, 3.62)	0.530	0.98 5
Hg	Model 1	-2.38 (-6.54, 1.95)	0.274	0.77 9	3.96 (-0.88, 9.03)	0.109	0.98 5
	Model 2	-2.46 (-6.58, 1.85)	0.257	0.77 9	3.35 (-1.50, 8.43)	0.177	0.98 5
	Model 3	-1.60 (-5.80, 2.79)	0.467	0.77 9	2.30 (-2.67, 7.54)	0.367	0.98 5
Ni	Model 1	1.51 (-2.91, 6.13)	0.506	0.77 9	4.86 (-1.20, 11.30)	0.117	0.98 5
	Model 2	1.56 (-2.84, 6.15)	0.491	0.77 9	4.09 (-1.97, 10.54)	0.188	0.98 5
	Model 3	2.11 (-2.52, 6.95)	0.375	0.77 9	3.08 (-3.02, 9.56)	0.326	0.98 5
Pb	Model 1	-2.16 (-7.11, 3.04)	0.406	0.77 9	-2.26 (-7.85, 3.66)	0.443	0.98 5

Sn	Model			0.77		0.98
	2	-2.09 (-7.01, 3.09)	0.418	9	-1.84 (-7.43, 4.09)	0.532
	Model			0.77		0.98
	3	-1.33 (-6.38, 4.00)	0.616	9	-2.13 (-7.86, 3.96)	0.481
	Model			0.99		0.98
	1	0.08 (-7.54, 8.33)	0.984	4	-3.33 (-9.59, 3.36)	0.318
TI	Model			0.99		0.98
	2	0.12 (-7.46, 8.32)	0.976	4	-2.87 (-9.14, 3.82)	0.388
	Model			0.99		0.98
	3	-0.17 (-7.86, 8.16)	0.966	4	-2.98 (-9.53, 4.05)	0.393
	Model			0.77		0.99
	1	-1.22 (-4.02, 1.65)	0.398	9	-0.30 (-3.90, 3.45)	0.874
Zn	Model			0.77		0.99
	2	-1.02 (-3.81, 1.86)	0.481	9	-0.32 (-3.90, 3.39)	0.861
	Model			0.77		0.98
	3	-0.78 (-3.63, 2.15)	0.596	9	-0.67 (-4.35, 3.15)	0.724
	Model			0.77		0.98
	1	2.43 (-2.45, 7.55)	0.332	9	-1.58 (-6.42, 3.51)	0.533
	Model			0.77		0.98
	2	2.60 (-2.26, 7.69)	0.297	9	-0.90 (-5.81, 4.26)	0.724
	Model			0.77		0.99
	3	2.88 (-2.05, 8.06)	0.255	9	-0.13 (-5.27, 5.29)	0.961

Notes: Sample sizes: males $n = 136$; females $n = 112$. Values represent percent change (95% CI) in AGD associated with an interquartile range increase in first-trimester plasma metal concentration. Models: Model 1 (Crude), unadjusted; Model 2 (Demographic), adjusted for child's age; Model 3 (Fully adjusted), adjusted for child's age, maternal age, gestational age, pre-pregnancy BMI, parity, maternal education, and household income. P values were calculated using linear regression. P_{FDR} values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) method, applied separately by outcome and sex. Statistical significance was defined as $P < 0.05$. Bold indicates $P < 0.05$ (or $P_{FDR} < 0.05$). All P values are two-sided. AGD, anogenital distance. Abbreviations: AGD, anogenital distance; AGD-AS, anogenital distance (anus-scrotum) for males; AGD-AF, anogenital distance (anus-fourchette) for females; BMI, body mass index; CI, confidence interval; FDR, false discovery rate; P_{FDR} , false discovery rate-adjusted P value; IQR, interquartile range.

Supplementary Table 5. Sex-stratified associations between first-trimester plasma metal concentrations and anogenital distance (AGD-AP/AGD-AC) in 2-year-old children

Metal	Model	AGD-AP (male)			AGD-AC (female)		
		Percent change (% 95% CI)	<i>P</i> value	<i>P</i> _{FD} <i>R</i>	Percent change (% 95% CI)	<i>P</i> value	<i>P</i> _{FD} <i>R</i>
As	Model 1	-2.64 (-4.54, -0.70)	0.008	0.03	-1.12 (-4.21, 2.08)	0.485	0.90
	Model 2	-2.63 (-4.54, -0.68)	0.009	0.03	-1.27 (-4.39, 1.95)	0.431	0.90
	Model 3	-2.83 (-4.80, -0.81)	0.007	0.03	-2.06 (-5.15, 1.12)	0.200	0.49
Cd	Model 1	-6.96 (-10.75, -3.01)	0.001	0.01	-0.49 (-7.00, 6.48)	0.886	0.98
	Model 2	-6.99 (-10.79, -3.03)	0.001	0.01	-0.94 (-7.56, 6.15)	0.786	0.95
	Model 3	-6.96 (-10.92, -2.82)	0.001	0.01	-1.37 (-7.85, 5.56)	0.687	0.95
Cr	Model 1	-3.21 (-5.74, -0.61)	0.016	0.04	4.01 (-0.03, 8.21)	0.052	0.27
	Model 2	-3.19 (-5.73, -0.58)	0.017	0.04	3.89 (-0.19, 8.15)	0.062	0.27
	Model 3	-3.19 (-5.88, -0.42)	0.025	0.05	2.89 (-1.04, 6.98)	0.150	0.49
Cu	Model 1	1.70 (-0.92, 4.39)	0.203	0.32	-1.27 (-5.93, 3.61)	0.600	0.90
	Model 2	1.64 (-1.02, 4.36)	0.227	0.32	-1.38 (-6.05, 3.52)	0.571	0.90
	Model 3	1.59 (-1.29, 4.56)	0.280	0.32	-1.37 (-5.87, 3.35)	0.560	0.90
Hg	Model 1	-1.48 (-3.98, 1.08)	0.252	0.32	4.27 (-0.24, 8.98)	0.064	0.27
	Model 2	-1.50 (-4.00, 1.07)	0.249	0.32	4.64 (0.05, 9.44)	0.047	0.27
	Model 3	-1.43 (-4.02, 1.23)	0.287	0.32	3.71 (-0.78, 8.40)	0.105	0.39
Ni	Model 1	0.15 (-2.45, 2.83)	0.909	0.90	8.09 (2.39, 14.10)	0.005	0.08
	Model 2	0.16 (-2.45, 2.84)	0.905	0.90	8.65 (2.85, 14.77)	0.003	0.08
	Model 3	0.54 (-2.27, 3.44)	0.706	0.75	6.94 (1.40, 12.79)	0.014	0.14
Pb	Model 1	-3.67 (-6.52, -0.73)	0.015	0.04	-0.07 (-5.42, 5.58)	0.979	0.98
				0			1

Sn	Model			0.04		0.98
	2	-3.66 (-6.52, -0.71)	0.016	0	-0.23 (-5.60, 5.44)	0.934
	Model			0.05		0.95
	3	-3.48 (-6.48, -0.39)	0.028	6	-0.60 (-5.84, 4.92)	0.825
	Model			0.32		0.90
	1	-2.63 (-7.06, 2.02)	0.261	4	2.28 (-3.92, 8.87)	0.477
Tl	Model			0.32		0.90
	2	-2.62 (-7.06, 2.04)	0.263	4	2.11 (-4.11, 8.74)	0.512
	Model			0.32		0.95
	3	-2.58 (-7.22, 2.29)	0.291	4	1.10 (-5.05, 7.65)	0.731
	Model			0.03		0.49
	1	-2.25 (-3.86, -0.62)	0.007	3	2.28 (-1.15, 5.82)	0.193
Zn	Model			0.03		0.49
	2	-2.23 (-3.85, -0.59)	0.008	3	2.29 (-1.15, 5.84)	0.192
	Model			0.04		0.86
	3	-2.16 (-3.85, -0.45)	0.014	0	1.52 (-1.84, 5.00)	0.376
	Model			0.09		0.95
	1	-2.79 (-5.52, 0.03)	0.052	7	-0.61 (-5.18, 4.19)	0.798
	Model			0.09		0.95
	2	-2.76 (-5.51, 0.06)	0.055	7	-0.88 (-5.51, 3.98)	0.715
	Model			0.10		0.98
	3	-2.78 (-5.64, 0.16)	0.063	6	-0.06 (-4.68, 4.78)	0.981

Notes: Sample sizes: males $n = 136$; females $n = 112$. Values represent percent change (95% CI) in AGD associated with an interquartile range increase in first-trimester plasma metal concentration. Models: Model 1 (Crude), unadjusted; Model 2 (Demographic), adjusted for child's age; Model 3 (Fully adjusted), adjusted for child's age, maternal age, gestational age, pre-pregnancy BMI, parity, maternal education, and household income. P values were calculated using linear regression. P_{FDR} values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) method, applied separately by outcome and sex. Statistical significance was defined as $P < 0.05$. Bold indicates $P < 0.05$ (or $P_{FDR} < 0.05$). All P values are two-sided. AGD, anogenital distance. Abbreviations: AGD, anogenital distance; AGD-AP, anogenital distance (anus-penis) for males; AGD-AC, anogenital distance (anus-clitoris) for females; BMI, body mass index; CI, confidence interval; FDR, false discovery rate; P_{FDR} , false discovery rate-adjusted P value; IQR, interquartile range.

As	4.45 (-5.86, 15.89)	0.408	-4.56 (-14.22, 6.19)	0.388	2.55 (-7.88, 14.17)	0.642	0.61 (-2.67, 4.00)	0.717
Cd	-0.66 (-9.45, 8.99)	0.888	-0.89 (-9.89, 9.02)	0.853	—	—	-4.69 (-42.36, 57.60)	0.850
Cr	-0.86 (-11.11, 10.57)	0.875	-0.17 (-10.50, 11.35)	0.975	-5.64 (-15.34, 5.17)	0.291	-8.40 (-21.33, 6.66)	0.255
Cu	-7.62 (-16.76, 2.52)	0.134	-4.16 (-13.70, 6.43)	0.423	-5.35 (-14.82, 5.17)	0.303	-0.01 (-0.02, 0.01)	0.465
Hg	2.61 (-7.86, 14.27)	0.635	-4.61 (-14.33, 6.22)	0.386	4.71 (-5.80, 16.38)	0.390	18.34 (-25.20, 87.23)	0.468
Ni	7.17 (-3.45, 18.97)	0.191	3.81 (-6.42, 15.16)	0.476	10.49 (-0.49, 22.67)	0.062	5.97 (-1.30, 13.77)	0.109
Pb	-8.41 (-17.86, 2.12)	0.112	-6.03 (-15.69, 4.74)	0.258	-3.92 (-13.65, 6.90)	0.459	-0.19 (-1.49, 1.12)	0.773
Sn	-6.58 (-14.88, 2.52)	0.149	-2.65 (-11.20, 6.73)	0.563	—	—	-25.86 (-63.94, 52.44)	0.412
Tl	-1.85 (-11.94, 9.40)	0.733	1.98 (-8.38, 13.51)	0.717	0.52 (-9.62, 11.81)	0.923	-17.80 (-74.18, 161.69)	0.738
Zn	0.32 (-9.84, 11.61)	0.953	-2.61 (-12.58, 8.50)	0.629	1.31 (-9.05, 12.84)	0.811	0.00 (-0.00, 0.01)	0.772

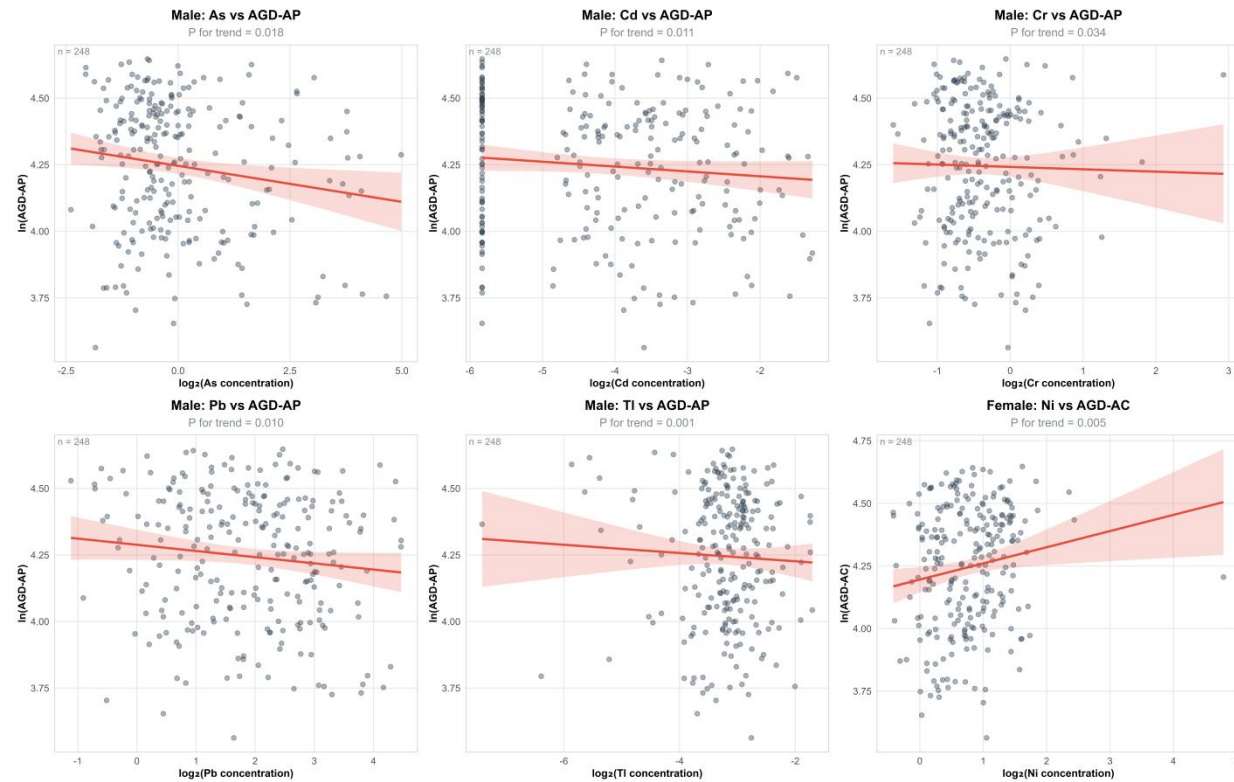
Notes: Sample sizes: males n = 136; females n = 112. Values are percent change (95% CI) in AGD-AS/AF per quartile increase in metal concentration, relative to Q1 (lowest quartile), estimated from fully adjusted linear regression models (Model 3) adjusting for child's age, maternal age, gestational age, pre-pregnancy BMI, parity, maternal education, and household income. *P* for trend was calculated using the median method: the median concentration of each quartile category was assigned as a continuous score and included as a continuous predictor in the fully adjusted model. “—” indicates not estimable due to an insufficient number of distinct quartile cutpoints, typically resulting from a high proportion of concentrations below the limit of detection. Bold indicates *P* < 0.05. All *P* values are two-sided. Abbreviations: AGD, anogenital distance; AGD-AS, anogenital distance (anus-scrotum) for males; AGD-AF, anogenital distance (anus-fourchette) for females; BMI, body mass index; CI, confidence interval; PBMI, pre-pregnancy body mass index; Q, quartile.

Supplementary Table 7. Quartile-based associations between first-trimester plasma metal concentrations and anogenital distance (AGD-AP/AGD-AC) in 2-year-old children using the fully adjusted model

Metals	Q2 vs Q1		Q3 vs Q1		Q4 vs Q1		P for trend	
	Percent change (%)	P value	Percent change (%)	P value	Percent change (%)	P value	Percent change (%)	P value
AGD-AP (male)								
As	0.74 (-5.28, 7.15)	0.812	-1.22 (-7.08, 5.01)	0.691	-5.85 (-11.51, 0.18)	0.057	-2.53 (-4.58, -0.44)	0.018
Cd	-3.78 (-8.70, 1.40)	0.149	-6.46 (-11.24, -1.41)	0.013	—	—	-33.64 (-51.59, -9.04)	0.011
Cr	1.37 (-4.80, 7.94)	0.669	-1.14 (-7.09, 5.21)	0.717	-5.62 (-11.42, 0.55)	0.073	-10.93 (-19.98, -0.86)	0.034
Cu	7.95 (1.51, 14.79)	0.015	6.53 (-0.19, 13.70)	0.057	5.27 (-1.35, 12.33)	0.120	0.01 (-0.00, 0.01)	0.230
Hg	-3.15 (-8.94, 3.02)	0.307	-4.10 (-9.96, 2.13)	0.190	-3.82 (-9.63, 2.37)	0.219	-14.98 (-36.00, 12.96)	0.261
Ni	8.78 (2.42, 15.53)	0.007	5.85 (-0.45, 12.54)	0.069	5.62 (-0.61, 12.23)	0.077	2.19 (-1.54, 6.06)	0.251
Pb	-2.64 (-8.38, 3.46)	0.386	-0.87 (-6.64, 5.25)	0.773	-7.76 (-13.24, -1.93)	0.010	-0.88 (-1.54, -0.21)	0.010
Sn	-2.40 (-8.28, 3.84)	0.439	-4.09 (-9.89, 2.07)	0.187	-3.83 (-9.60, 2.32)	0.215	-28.62 (-56.19, 16.30)	0.174
Tl	3.43 (-2.38, 9.59)	0.251	0.17 (-5.55, 6.24)	0.954	-9.04 (-14.20, -3.57)	0.002	-68.51 (-83.04, -41.54)	< 0.001
Zn	-2.87 (-8.58, 3.20)	0.343	-6.26 (-11.79, -0.38)	0.037	-6.04 (-11.62, -0.10)	0.046	-0.00 (-0.01, 0.00)	0.106
AGD-AC (female)								

As	13.08 (3.36, 23.72)	0.008	9.07 (-0.54, 19.61)	0.065	0.75 (-8.18, 10.54)	0.874	-1.84 (-4.68, 1.09)	0.214
Cd	-0.79 (-8.66, 7.77)	0.850	-3.39 (-11.25, 5.18)	0.423	—	—	-16.64 (-46.77, 30.54)	0.423
Cr	3.32 (-6.11, 13.70)	0.500	9.68 (-0.34, 20.70)	0.059	-1.40 (-10.35, 8.45)	0.770	-3.30 (-15.67, 10.89)	0.628
Cu	-2.81 (-11.53, 6.78)	0.549	-1.27 (-10.19, 8.53)	0.789	-2.39 (-11.25, 7.36)	0.616	-0.00 (-0.02, 0.01)	0.719
Hg	1.12 (-8.14, 11.30)	0.819	1.00 (-8.23, 11.17)	0.837	8.77 (-1.01, 19.52)	0.080	46.77 (-2.07, 119.96)	0.063
Ni	7.55 (-1.70, 17.68)	0.111	2.65 (-6.13, 12.24)	0.563	15.92 (5.93, 26.85)	0.002	9.46 (2.89, 16.45)	0.005
Pb	1.35 (-8.13, 11.81)	0.787	3.24 (-6.39, 13.87)	0.520	-1.26 (-10.33, 8.73)	0.794	-0.16 (-1.32, 1.02)	0.789
Sn	2.84 (-5.43, 11.84)	0.509	1.75 (-6.34, 10.55)	0.678	—	—	18.29 (-38.01, 125.69)	0.607
Tl	9.75 (-0.01, 20.46)	0.050	8.62 (-1.11, 19.30)	0.083	3.63 (-5.76, 13.96)	0.458	28.10 (-54.51, 260.74)	0.636
Zn	-1.97 (-10.84, 7.78)	0.678	4.57 (-5.01, 15.11)	0.358	-0.18 (-9.30, 9.86)	0.971	0.00 (-0.00, 0.00)	0.926

Notes: Sample sizes: males n = 136; females n = 112. Values are percent change (95% CI) in AGD-AP/AC per quartile increase in metal concentration, relative to Q1 (lowest quartile), estimated from fully adjusted linear regression models (Model 3) adjusting for child's age, maternal age, gestational age, pre-pregnancy BMI, parity, maternal education, and household income. *P* for trend was calculated using the median method: the median concentration of each quartile category was assigned as a continuous score and included as a continuous predictor in the fully adjusted model. “—” indicates not estimable due to an insufficient number of distinct quartile cutpoints, typically resulting from a high proportion of concentrations below the limit of detection. Bold indicates *P* < 0.05. All *P* values are two-sided. AGD, anogenital distance; AGD-AP, anogenital distance (anus-penis) for males; AGD-AC, anogenital distance (anus-clitoris) for females; BMI, body mass index; CI, confidence interval; PBMI, pre-pregnancy body mass index; Q, quartile.



Supplementary Figure 3. Dose-response relationships between first-trimester plasma metal concentrations and AGD-AP/AC in 2-year-old children. Sample sizes: males $n = 136$; females $n = 112$. Panels show scatter plots of log₂-transformed metal concentrations against natural log-transformed AGD-AP (males: As, Cd, Cr, Pb, Tl) or natural log-transformed AGD-AC (females: Ni). Solid red lines represent linear regression fits with 95% confidence bands (shaded areas). P for trend values, calculated by assigning the median concentration of each quartile category as a continuous score in fully adjusted linear regression models (Model 3), are shown in each panel. Statistical significance was defined as $P < 0.05$. Only metals with P for trend < 0.05 are shown. Shaded areas represent 95% confidence intervals. Abbreviations: CI, confidence interval; PBMI, pre-pregnancy body mass index; AGD, anogenital distance; AGD-AP, anopenile distance in males; AGD-AC, anoclitral distance in females.

Supplementary Table 8. Metal × sex interaction effects on AGD measures

Metal	Interaction Estimate (95% CI)	<i>P</i> value
AGD- AP/AC		
Cr	0.0605 (0.0226, 0.0984)	0.002
Ni	0.0533 (0.0127, 0.0940)	0.011
Tl	0.0498 (0.0099, 0.0898)	0.015
Hg	0.0465 (0.0079, 0.0851)	0.019
Cd	0.0326 (-0.0058, 0.0709)	0.097
Pb	0.0274 (-0.0118, 0.0667)	0.172
Sn	0.0264 (-0.0124, 0.0651)	0.184
Cu	-0.0243 (-0.0640, 0.0154)	0.231
Zn	0.0206 (-0.0185, 0.0598)	0.303
As	0.0121 (-0.0272, 0.0514)	0.546
AGD- AS/AF		
Hg	0.0518 (-0.0004, 0.1040)	0.053
Cu	-0.0382 (-0.0915, 0.0151)	0.162
Zn	-0.0343 (-0.0872, 0.0185)	0.204
Ni	0.0276 (-0.0280, 0.0831)	0.332
Sn	-0.0177 (-0.0699, 0.0346)	0.509
Cr	0.0134 (-0.0385, 0.0654)	0.613
Tl	0.0096 (-0.0447, 0.0640)	0.729
Cd	-0.0072 (-0.0595, 0.0452)	0.789
Pb	-0.0037 (-0.0569, 0.0495)	0.892
As	-0.0011 (-0.0545, 0.0522)	0.967

Notes: Interaction estimates were derived from fully adjusted linear regression models including a metal × sex interaction term. Positive estimates indicate stronger associations in females than in males; negative estimates indicate stronger associations in males than in females. *P* values are from the interaction term (metal × sex). Bold indicates *P* < 0.05. No interactions were significant for AGD- AS/AF (all *P* > 0.05). N = 248 for all models. Abbreviations: CI, confidence interval; AGD-AP/AC: anopenile distance in males/anoclitral distance in females. AGD-AS/AF: anoscrotal distance in males/anofourchette distance in fema

Supplementary Table 9. Sensitivity analysis for the association between each metal and AGD-AP (males) / AGD-AC (females): comparison of models with and without adjustment for birth weight

Sex	Metal	Main (no BW)		Adjust BW	
		β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>
Male	Cr	-0.0264 (-0.0491, -0.0036)	0.025	-0.0265 (-0.0493, -0.0037)	0.024
	Ni	0.0039 (-0.0165, 0.0243)	0.706	0.0033 (-0.0174, 0.0239)	0.758
	Cu	0.0121 (-0.0097, 0.0338)	0.279	0.0113 (-0.0110, 0.0336)	0.324
	Zn	-0.0207 (-0.0423, 0.0010)	0.063	-0.0209 (-0.0426, 0.0008)	0.061
	As	-0.0305 (-0.0521, -0.0089)	0.007	-0.0302 (-0.0520, -0.0085)	0.007
	Cd	-0.0358 (-0.0572, -0.0144)	0.001	-0.0355 (-0.0571, -0.0140)	0.002
	Sn	-0.0130 (-0.0372, 0.0111)	0.291	-0.0128 (-0.0370, 0.0115)	0.304
	Hg	-0.0114 (-0.0324, 0.0095)	0.288	-0.0108 (-0.0321, 0.0104)	0.319
	Tl	-0.0249 (-0.0446, -0.0053)	0.014	-0.0248 (-0.0446, -0.0051)	0.015
	Pb	-0.0234 (-0.0441, -0.0028)	0.028	-0.0234 (-0.0441, -0.0026)	0.029
Female	Cr	0.0232 (-0.0082, 0.0546)	0.150	0.0232 (-0.0082, 0.0547)	0.151
	Ni	0.0487 (0.0105, 0.0868)	0.014	0.0482 (0.0099, 0.0866)	0.015
	Cu	-0.0105 (-0.0457, 0.0247)	0.560	-0.0093 (-0.0450, 0.0264)	0.610
	Zn	-0.0004 (-0.0347, 0.0338)	0.981	-0.0003 (-0.0347, 0.0340)	0.985
	As	-0.0222 (-0.0558, 0.0115)	0.200	-0.0217 (-0.0556, 0.0121)	0.211
	Cd	-0.0069 (-0.0402, 0.0265)	0.687	-0.0066 (-0.0400, 0.0269)	0.701
	Sn	0.0055 (-0.0255, 0.0364)	0.731	0.0061 (-0.0250, 0.0373)	0.701
	Hg	0.0290 (-0.0058, 0.0637)	0.105	0.0286 (-0.0064, 0.0635)	0.112
	Tl	0.0172 (-0.0207, 0.0551)	0.376	0.0168 (-0.0212, 0.0549)	0.388
	Pb	-0.0040 (-0.0393, 0.0313)	0.825	-0.0034 (-0.0389, 0.0322)	0.853

Notes: Sample sizes: males $n = 136$; females $n = 112$. The "Main (no BW)" model includes all covariates from Model 3 except birth weight; the "Adjust BW" model includes birth weight as an additional covariate. The near-identical estimates between the two models indicate that adjustment for birth weight does not materially alter the associations, suggesting that birth weight is not a major confounder or mediator in these relationships. Sensitivity analyses were performed separately by sex and for each metal. Statistically significant associations ($P < 0.05$) are indicated in bold. Abbreviations: AGD, anogenital distance; AGD-AP, anogenital distance (anus-penis) for males; AGD-AC, anogenital distance (anus-clitoris) for females; BW, birth weight; CI, confidence interval; β , standardized regression coefficient.

Supplementary Table 10. Sensitivity analysis for extreme value treatment: truncation and winsorization of metal concentrations (5th–95th percentiles)

Sex	Metal	Main model		Truncate 5-95%		Winsorize 5-95%	
		Beta (95% CI)	<i>P</i>	Beta (95% CI)	<i>P</i>	Beta (95% CI)	<i>P</i>
Male	Cr	-0.0264 (-0.0491, -0.0036)	0.025	-0.0320 (-0.0675, 0.0035)	0.080	-0.0313 (-0.0592, -0.0034)	0.030
	Ni	0.0039 (-0.0165, 0.0243)	0.706	0.0108 (-0.0184, 0.0400)	0.470	0.0160 (-0.0097, 0.0417)	0.225
	Cu	0.0121 (-0.0097, 0.0338)	0.279	0.0261 (-0.0060, 0.0582)	0.114	0.0185 (-0.0067, 0.0438)	0.153
	Zn	-0.0207 (-0.0423, 0.0010)	0.063	-0.0211 (-0.0514, 0.0093)	0.177	-0.0224 (-0.0466, 0.0019)	0.073
	As	-0.0305 (-0.0521, -0.0089)	0.007	-0.0146 (-0.0455, 0.0164)	0.358	-0.0306 (-0.0545, -0.0067)	0.013
	Cd	-0.0358 (-0.0572, -0.0144)	0.001	-0.0379 (-0.0609, -0.0149)	0.002	-0.0374 (-0.0592, -0.0155)	0.001
	Sn	-0.0130 (-0.0372, 0.0111)	0.291	-0.0153 (-0.0426, 0.0120)	0.274	-0.0137 (-0.0392, 0.0118)	0.293
	Hg	-0.0114 (-0.0324, 0.0095)	0.287	-0.0172 (-0.0475, 0.0132)	0.270	-0.0155 (-0.0400, 0.0090)	0.217
	Tl	-0.0249 (-0.0446, -0.0053)	0.014	-0.0396 (-0.0776, -0.0015)	0.044	-0.0377 (-0.0637, -0.0118)	0.005
	Pb	-0.0234 (-0.0441, -0.0028)	0.028	-0.0152 (-0.0429, 0.0125)	0.284	-0.0239 (-0.0459, -0.0020)	0.034
Female	Cr	0.0232 (-0.0082, 0.0546)	0.150	-0.0267 (-0.0751, 0.0216)	0.282	0.0077 (-0.0333, 0.0487)	0.713
	Ni	0.0487 (0.0105, 0.0868)	0.014	0.0784 (0.0307, 0.1261)	0.002	0.0551 (0.0139, 0.0964)	0.010
	Cu	-0.0105 (-0.0457, 0.0247)	0.560	0.0315 (-0.0191, 0.0821)	0.225	-0.0093 (-0.0484, 0.0298)	0.642

Zn	-0.0004 (-0.0347, 0.0338)	0.981	0.0296 (-0.0140, 0.0731)	0.186	0.0004 (-0.0367, 0.0375)	0.983
As	-0.0222 (-0.0558, 0.0115)	0.200	-0.0210 (-0.0638, 0.0219)	0.340	-0.0210 (-0.0573, 0.0152)	0.258
Cd	-0.0069 (-0.0402, 0.0265)	0.687	-0.0012 (-0.0376, 0.0353)	0.951	-0.0062 (-0.0400, 0.0276)	0.719
Sn	0.0055 (-0.0255, 0.0364)	0.731	0.0176 (-0.0235, 0.0587)	0.403	0.0122 (-0.0240, 0.0484)	0.512
Hg	0.0290 (-0.0058, 0.0637)	0.105	0.0583 (0.0123, 0.1043)	0.015	0.0335 (-0.0035, 0.0706)	0.079
Tl	0.0172 (-0.0207, 0.0551)	0.376	0.0155 (-0.0518, 0.0828)	0.653	0.0186 (-0.0329, 0.0701)	0.481
Pb	-0.0040 (-0.0393, 0.0313)	0.825	0.0035 (-0.0390, 0.0459)	0.874	-0.0046 (-0.0420, 0.0329)	0.811

Notes: Three approaches were compared: (1) Main model (no extreme value treatment); (2) Truncate 5-95% (excluded observations below 5th or above 95th percentile); (3) Winsorize 5-95% (replaced extremes with 5th/95th percentile values). β coefficients represent percent change in AGD-AP (males) or AGD-AC (females) per IQR increase. All models were adjusted for the same covariates as Model 3: child's age, maternal age, gestational age, PBMI, parity, maternal education, and household income. N_Main = 136 for males and 112 for females, representing the full analytical sample before extreme value treatment. N_Truncate = 122 for males and 100 for females, reflecting the exclusion of observations below the 5th or above the 95th percentile of each metal concentration. N_Winsorize = 136 for males and 112 for females, equal to the full sample size because winsorization replaces extreme values rather than excluding observations. Abbreviations: AGD, anogenital distance; AGD-AP, anogenital distance (anus-penis) for males; AGD-AC, anogenital distance (anus-clitoris) for females; CI, confidence interval; IQR, interquartile range; PBMI, pre-pregnancy body mass index; β , standardized regression coefficient; ; N, number of participants.