Supplementary Materials

Title: Urinary polycyclic aromatic hydrocarbon metabolites and Crohn's disease activity: the mediating role of oxidative stress

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Table S1. Crohn's disease Harvey-Bradshaw index (HBI) [1]

Parameters	0	1	2	3	4
General wellbeing	Very well	Slightly below par	Poor	Very poor	Terrible
Abdominal pain	None	Mild	Moderate	Severe	-
Abdominal mass	None	Dubious	Definite	Definite and tender	-
Number of liquid stools per day	Score 1 per liquid stool				
Complications [a]	Score 1 per complication				

Note: [a] Complications include arthralgia, uveitis, erythema nodosum, aphthous ulcers, pyoderma gangrenosum, anal fissure, new fistula, abscess, etc.

Table S2. Simplified endoscopic score for Crohn's disease (SES-CD) [2]

Parameters	0	1	2	3
Ulcer size	None	Aphthous ulcers (Ø 0.1-0.5 cm)	Large ulcers (Ø 0.5-2.0 cm)	Very large ulcers (Ø > 2.0 cm)
Ulcerated surface	None	< 10%	10-30%	> 30%
Affected surface	None	< 50%	50-75%	> 75%
Stenosis	None	Single, can be passed	Multiple, can be passed	Cannot be passed

Note: Ø, diameter.

Intestinal segment	Bowel wall thickness	Mural hyper-enhancement	Mural stratification	Engorged vasa recta
Ileum	Yes/No = 1/0	Yes/No = 1/0	Yes/No = 1/0	Yes/No = 1/0
Right colon	Yes/No = 1/0	Yes/No = 1/0	Yes/No = 1/0	Yes/No = 1/0
Transverse colon	Yes/No = 1/0	Yes/No = 1/0	Yes/No = 1/0	Yes/No = 1/0
Sigmoid and left colon	Yes/No = 1/0	Yes/No = 1/0	Yes/No = 1/0	Yes/No = 1/0
Rectum	Yes/No = 1/0	Yes/No = 1/0	Yes/No = 1/0	Yes/No = 1/0

Table S3. Computed tomography enterography score (CTE) for Crohn's disease[3]

Table S4. Residuals normality test of regression models by Shapiro-Wilk tests

	HBI	BMI	SES-CD	CTE	8-OHdG	8-iso-PGF2α
1-OHNa	0.115	0.844	0.327	0.017	0.760	0.590
2-OHNa	0.157	0.900	0.226	0.008	0.517	0.814
1-OHPh	0.292	0.949	0.147	0.004	0.371	0.149
2-OHPh	0.109	0.853	0.081	0.005	0.796	0.477
3-OHPh	0.259	0.955	0.096	0.016	0.106	0.522
4-OHPh	0.357	0.935	0.090	0.007	0.636	0.075
9-OHPh	0.363	0.945	0.073	0.009	0.385	0.296
2-OHFlu	0.199	0.777	0.067	0.005	0.683	0.875
9-OHFlu	0.095	0.854	0.165	0.005	0.021	0.246
1-OHP	0.093	0.898	0.314	0.004	0.491	0.647
ΣOHNa	0.164	0.891	0.250	0.011	0.705	0.816
ΣOHPh	0.326	0.888	0.072	0.010	0.226	0.163
ΣOHFlu	0.336	0.667	0.082	0.006	0.187	0.786
ΣOH-PAHs	0.140	0.885	0.344	0.008	0.643	0.529

Table S5. Linear regression and robust regression models on PAEs and CTE

	Linear regression	**Robust regression** [a]	**Robust regression** [b]
1-OHNa	0.085	0.063	0.161
2-OHNa	0.429	0.407	0.378
1-OHPh	0.272	0.216	0.172
2-OHPh	0.685	0.691	0.658
3-OHPh	0.211	0.190	0.304
4-OHPh	0.739	0.734	0.645
9-OHPh	0.273	0.240	0.319
2-OHFlu	0.971	0.932	0.626
9-OHFlu	0.566	0.480	0.430
1-OHP	0.102	0.084	0.100
ΣOHNa	0.243	0.217	0.245
ΣOHPh	0.196	0.193	0.235
ΣOHFlu	0.818	0.805	0.925
ΣOH-PAHs	0.660	0.663	0.623

a: based on Huber–White Sandwich Estimator

b: based on MM-estimator

Table S6. Percent changes (95% CI) of CD activity and oxidative stress in relation to urinary OH-PAHs in participants with all four CD indices (n=81)

OH-PAHs	Percent changes (95% CI)					
	HBI	BMI	SES-CD	CTE	8-OHdG	8-iso-PGF2α
1-OHNa	-2.1 (-11.8, 8.7)	-1.3 (-4.1, 1.5)	-9.4 (-22.7, 6.1)	6.7 (-3.7, 18.3)	30.6 (14.9, 48.3)	27.9 (12.2, 45.8)
2-OHNa	-6.5 (-16.5, 4.7)	0.6 (-2.5, 3.7)	-12.0 (-25.8, 4.5)	3.0 (-8.0, 15.3)	30.9 (13.7, 50.8)	29.5 (12.2, 49.5)
1-OHPh	7.3 (-3.9, 19.8)	-3.1 (-5.9, -0.2)	-6.9 (-21.4, 10.3)	6.7 (-4.3, 19.2)	54.6 (38.1, 73.3)	56.5 (40.0, 75.0)
2-OHPh	1.2 (-6.9, 9.9)	-0.6 (-2.8, 1.7)	2.6 (-9.6, 16.4)	-0.7 (-8.5, 7.8)	16.7 (4.9, 29.9)	16.5 (4.6, 29.7)
3-OHPh	14.9 (2.2, 29.1)	-3.0 (-6.0, 0.2)	-1.4 (-18.0, 18.5)	7.3 (-4.7, 20.8)	61.1 (42.8, 81.9)	56.4 (37.6, 77.8)
4-OHPh	17.9 (4.8, 32.8)	-5.1 (-8.0, -2.0)	-3.1 (-19.9, 17.2)	0.1 (-11.5, 13.4)	76.9 (59.1, 96.6)	75.9 (57.8, 96.2)
9-OHPh	9.7 (-2.5, 23.5)	-3.6 (-6.6, -0.6)	1.3 (-15.7, 21.7)	5.3 (-6.5, 18.4)	62.6 (44.5, 82.9)	65.5 (47.6, 85.6)
2-OHFlu	16.7 (2.7, 32.6)	-4.6 (-7.9, -1.2)	2.5 (-16.3, 25.5)	-0.7 (-12.9, 13.3)	53.5 (32.0, 78.4)	49.3 (27.8, 74.4)
9-OHFlu	1.0 (-10.5, 14.0)	-0.9 (-4.1, 2.5)	-9.2 (-24.5, 9.1)	5.3 (-6.6, 18.6)	45.5 (26.5, 67.2)	45.2 (26.1, 67.1)
1-OHP	3.1 (-1.5, 8.0)	-0.8 (-2.1, 0.4)	-2.7 (-9.3, 4.5)	-3.0 (-7.3, 1.5)	5.9 (-0.4, 12.7)	4.2 (-2.1, 11.0)
ΣOHNa	-5.1 (-15.4, 6.7)	-0.2 (-3.3, 3.0)	-12.1 (-26.3, 4.8)	4.8 (-6.6, 17.6)	36.6 (18.6, 57.4)	34.4 (16.4, 55.2)
ΣOHPh	14.8 (0.3, 31.4)	-4.0 (-7.5, -0.5)	-1.0 (-19.9, 22.5)	5.9 (-7.7, 21.6)	83.0 (61.2, 107.8)	81.8 (59.5, 106.9)
ΣOHFlu	15.0 (-0.8, 33.4)	-4.9 (-8.6, -1.0)	-3.1 (-23.1, 22.3)	2.2 (-12.0, 18.8)	84.8 (59.2, 114.5)	80.5 (54.4, 110.8)
ΣOH-PAHs	8.7 (-4.8, 24.1)	-3.5 (-6.8, 0.1)	-8.9 (-25.7, 11.7)	1.8 (-10.9, 16.4)	63.0 (41.3, 88.2)	57.0 (35.1, 82.6)

Note: Multivariate linear regression models were adjusted for age, gender, smoking status, drinking status and biological agents use. Urinary OH-PAHs were included as continuous variables.

Table S7. Percent changes (95% CI) of CD activity in relation to urinary oxidative stress biomarkers in participants with all four CD indices (n=81)

Oxidative stress biomarkers	Percent changes (95% CI)			
	HBI	BMI	SES-CD	CTE
8-OHdG	22.5 (10.0, 36.5)	-5.1 (-7.9, -2.3)	8.7 (-9.1, 30.1)	7.8 (-3.9, 21.2)
8-iso-PGF2α	20.2 (7.8, 34.2)	-4.7 (-7.5, -1.9)	11.2 (-7.0, 32.9)	9.0 (-2.9, 22.3)

Note: Multivariate linear regression models were adjusted for age, gender, smoking status, drinking status and biological agents use. Urinary oxidative stress biomarkers were included as continuous variables.

Table S8. Mediation effect of oxidative stress on the associations of urinary OH-PAHs with HBI and BMI in participants with all four CD indices (n=81)

Parameters	8-OHdG			8-iso-PGF2α		
	Indirect effect	Direct effect	Proportion of mediation (%)	Indirect effect	Direct effect	Proportion of mediation (%)
HBI	β (95% CI)	β (95% CI)		β (95% CI)	β (95% CI)	
3-OHPh	0.203 (0.056, 0.365)	-0.003 (-0.221, 0.214)	-	0.153 (0.013, 0.307)	0.047 (-0.163, 0.257)	76.5%
4-OHPh	0.246 (0.031, 0.436)	-0.007 (-0.273, 0.258)	-	0.186 (-0.029, 0.368)	0.053 (-0.211, 0.317)	77.8%
2-OHFlu	0.163 (0.047, 0.309)	0.060 (-0.151, 0.271)	73.1%	0.129 (0.019, 0.253)	0.094 (-0.114, 0.302)	57.8%
ΣOHPh	0.324 (0.144, 0.541)	-0.125 (-0.399, 0.149)	-	0.263 (0.081, 0.469)	-0.064 (-0.338, 0.211)	-
BMI	β (95% CI)	β (95% CI)		β (95% CI)	β (95% CI)	
1-OHPh	-0.050 (-0.083, -0.012)	0.004 (-0.050, 0.059)	-	-0.045 (-0.083, -0.005)	-0.001 (-0.057, 0.056)	97.8%
4-OHPh	-0.044 (-0.102, 0.011)	-0.030 (-0.103, 0.042)	59.5%	-0.031 (-0.091, 0.027)	-0.043 (-0.115, 0.028)	41.9%
9-OHPh	-0.054 (-0.095, -0.008)	0.001 (-0.060, 0.062)	-	-0.049 (-0.096, 0.003)	-0.004 (-0.068, 0.059)	92.5%
2-OHFlu	-0.038 (-0.072, -0.004)	-0.030 (-0.087, 0.028)	55.9%	-0.031 (-0.065, 0.002)	-0.037 (-0.094, 0.019)	45.6%
ΣOHPh	-0.075 (-0.124, -0.020)	0.016 (-0.060, 0.092)	-	-0.061 (-0.116, -0.002)	0.002 (-0.074, 0.077)	-
ΣOHFlu	-0.063 (-0.110, -0.004)	-0.009 (-0.086, 0.067)	87.5%	-0.049 (-0.102, 0.008)	-0.023 (-0.098, 0.052)	68.1%

Note: Proportion of mediation (%) = 100% × indirect effect / (indirect effect + direct effect). Models were adjusted for age, gender, smoking status, drinking status and biological agents use.

Table S9. Basic characteristics of healthy controls (HC) and CD patients

Characteristics	HC (n=48)	CD (n=127)	*P*
Age (years), mean ± SD	31.5 ± 10.3	29.5 ± 11.3	0.301
Gender, n (%)			0.935
Male	36 (75.0%)	96 (75.6%)	
Female	12 (25.0%)	31 (24.4%)	
Smoking status, n (%)			1.000
Nonsmoker	44 (91.7%)	117 (92.1%)	
Current/Former smoker	4 (8.3%)	10 (7.9%)	
Drinking status, n (%)			1.000
Nondrinker	47 (97.9%)	123 (96.9%)	
Current/Former drinker	1 (2.1%)	4 (3.1%)	
Biological agents use, n (%)			<0.001
No	48 (100.0%)	56 (44.1%)	
Yes	0 (0.0%)	71 (55.9%)	
BMI (kg/m^2), median (IQR)	22.5 (20.7, 24.7)	18.8 (16.4, 20.6)	<0.001

Note: Normal and non-normal continuous variables were presented as means ± standard deviation (SD) and medians (interquartile range, IQR), respectively, and differences were analyzed by Student's t-tests or Mann-Whitney U tests. Categorical variables were presented as percentages (%), and differences were analyzed by χ^2 tests.

Table S10. Descriptive statistics of urinary OH-PAHs in healthy controls (HC) and CD patients

Analytes (μg/L)	HC (n=48)			CD (n=127)			*P*
	Percentile			Percentile			
	25th	50th	75th	25th	50th	75th	
OH-PAHs							
1-OHNa	0.87	1.76	4.91	0.32	0.54	0.85	<0.001
2-OHNa	0.83	2.16	6.00	0.87	1.38	2.69	0.151
1-OHPh	0.04	0.08	0.16	0.11	0.17	0.21	<0.001
2-OHPh	0.10	0.30	0.80	0.22	0.35	0.69	0.630
3-OHPh	0.21	0.30	0.42	0.18	0.26	0.37	0.143
4-OHPh	0.08	0.16	0.17	0.07	0.09	0.16	0.023
9-OHPh	0.20	0.34	0.55	0.32	0.37	0.43	0.554
2-OHFlu	0.32	0.78	1.66	0.38	0.51	0.78	0.071
9-OHFlu	0.25	0.38	1.03	0.44	0.61	0.87	0.081
1-OHP	0.27	1.17	4.66	0.07	1.25	2.71	0.008

Note: Differences of urinary OH-PAHs were analyzed by Mann-Whitney U tests.

Table S11. Spearman correlation coefficients between CD activity indices

	HBI	BMI	SES-CD	CTE
HBI	**1**	**-0.416**	**0.442**	**0.279**
BMI		**1**	**-0.292**	**-0.256**
SES-CD			**1**	**0.407**
CTE				**1**

Note: HBI, Crohn's disease Harvey-Bradshaw index; BMI, body mass index; SES-CD, simple endoscopic score for Crohn's disease; CTE, computed tomography enterography score. All disease indices were normalized by $\log_2$-transformation. Coefficients in boldface indicate $P < 0.05$.

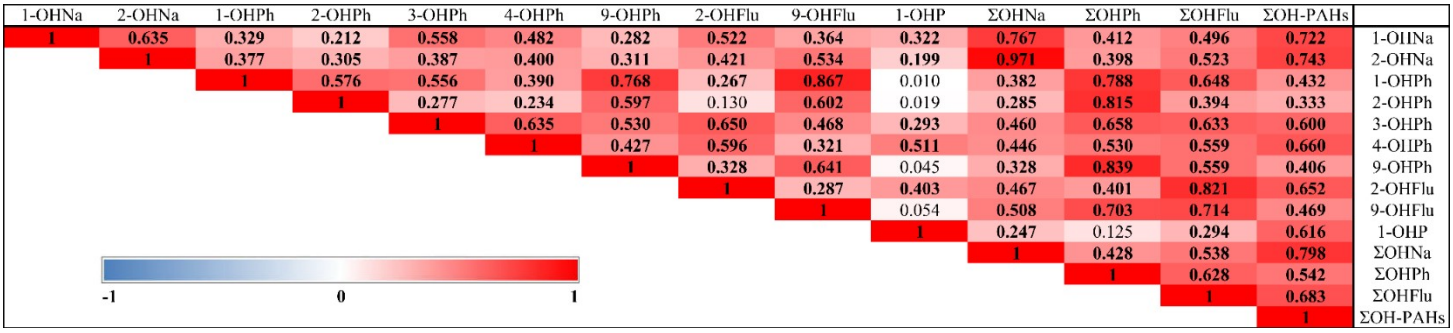

Figure S1. Spearman correlation coefficients between urinary OH-PAHs in CD patients. 1-OHNa, 1-hydroxynaphthalene; 2-OHNa, 2-hydroxynaphthalene; 1-OHPh, 1-hydroxyphenanthrene; 2-OHPh, 2-hydroxyphenanthrene; 3-OHPh, 3-hydroxyphenanthrene; 4-OHPh, 4-hydroxyphenanthrene; 9-OHPh, 9-hydroxyphenanthrene; 2-OHFlu, 2-hydroxyfluorene; 9-OHFlu, 9-hydroxyfluorene; 1-OHP, 1-hydroxypyrene; ΣOHNa, the sum of 1-OHNa and 2-OHNa; ΣOHPh, the sum of 1-OHPh, 2-OHPh, 3-OHPh, 4-OHPh and 9-OHPh; ΣOHFlu, the sum of 2-OHFlu and 9-OHFlu; ΣOH-PAHs, the sum of all ten monohydroxylated polycyclic aromatic hydrocarbons. Concentrations of urinary OH-PAHs were normalized by $\log_2$-transformation. Coefficients in boldface indicate $P < 0.05$.

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