

BDNF as a potential mediator between childhood BPA exposure and behavioral function in adolescent boys from the INMA-Granada cohort

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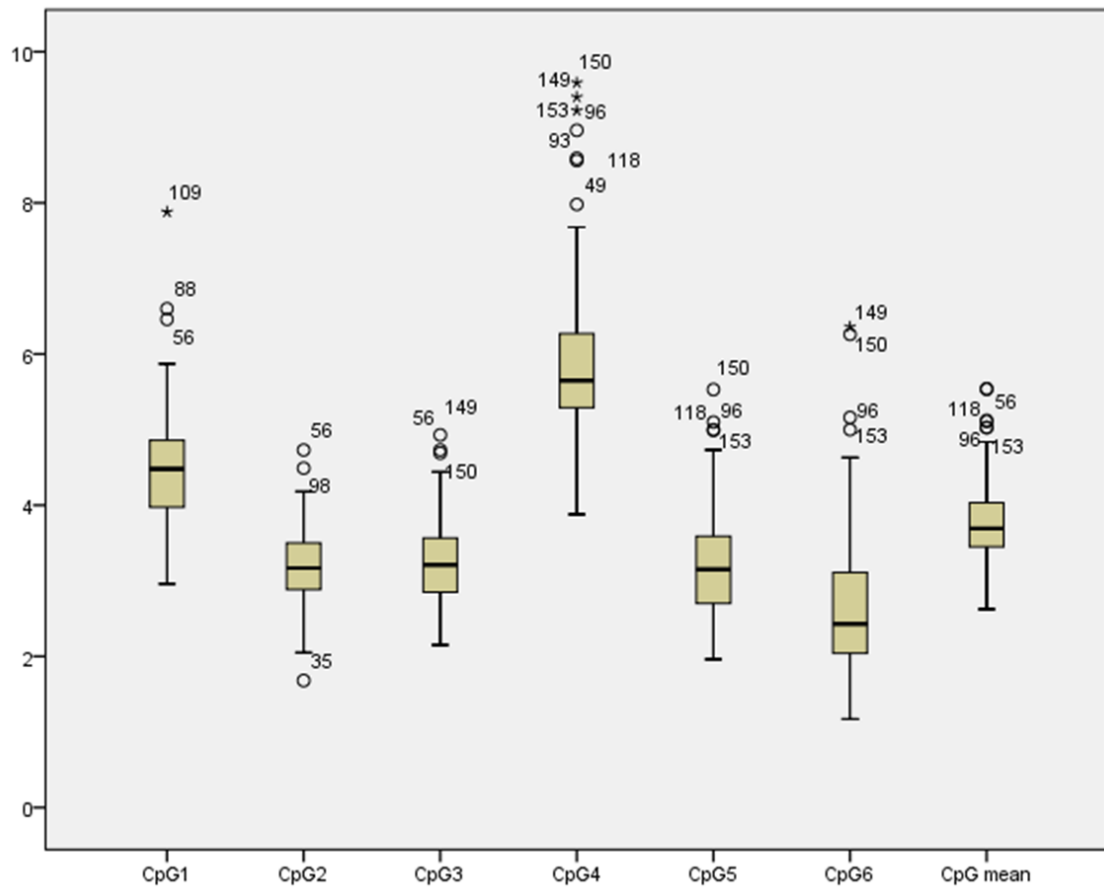
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Figure S1. Boxplot of BDNF DNA methylation at Exon IV of six CpG islands and the mean methylation of CpGs. The Y-axis presents the percentage of DNA methylation.



Note: Circles and stars represent outliers.

Figure S2. Tertile analysis of selected associations among BPA, BDNF DNA methylation and thought problems t-scores. Brown color (BPA-thought problems); blue color (BPA-BDNF methylation); and green color (BDNF methylation-thought problems).

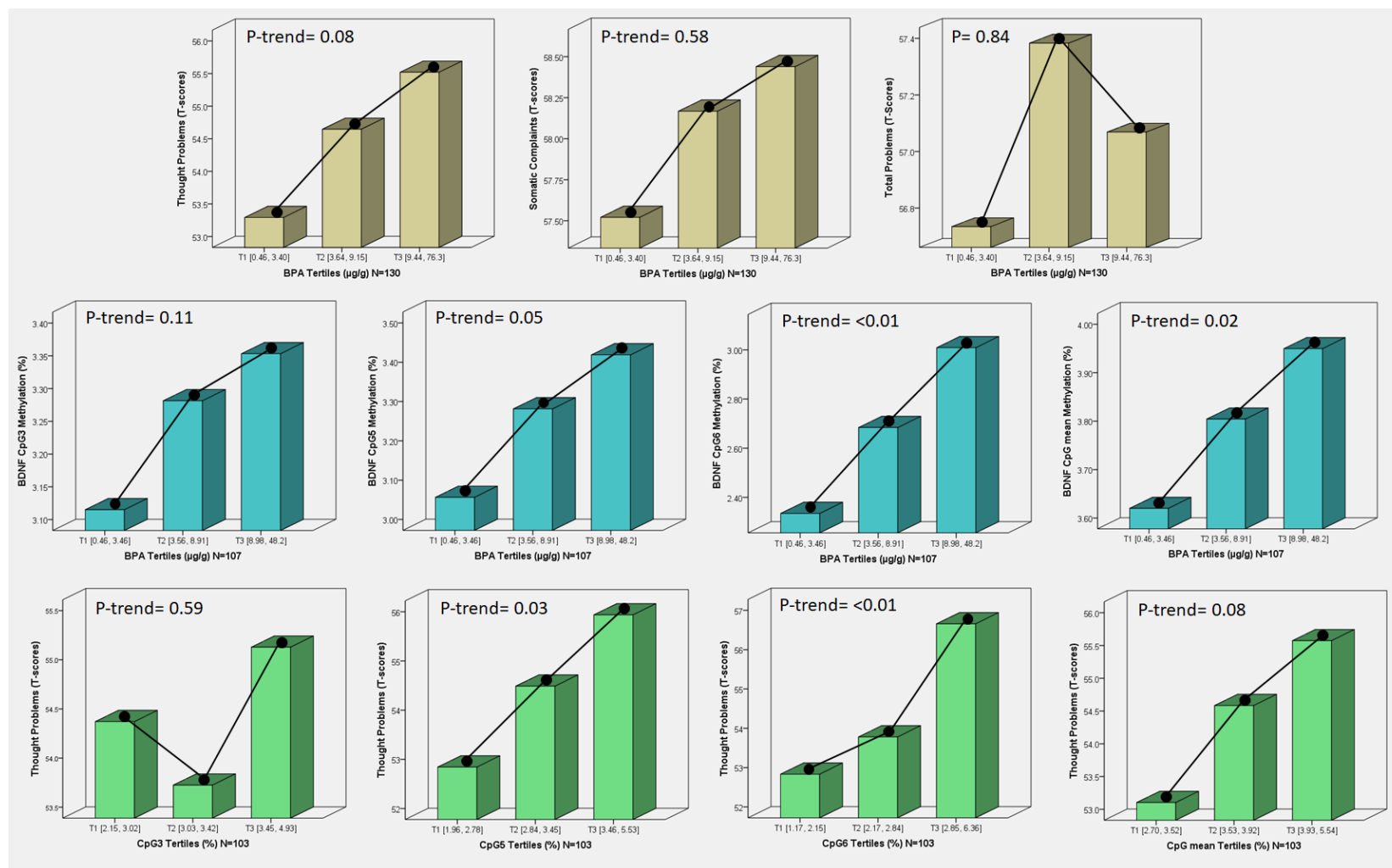


Table S1. Comparison of characteristics between the final study population and boys that participated in the previous follow-up at 9-11 years of age.

Characteristics	Study population with BPA at 9-11 y. and behavior data at 15-17yrs. (n=130)	Rest of sample with BPA at 9-11 yrs. that did not attend the latest follow-up (n=139)	P-value ^a
Child BPA concentrations (µg/g)	5.41 (3.05, 10.6)	4.41 (2.46, 9.06)	0.072
Child age (yrs)	9.82 (9.67, 10.0)	9.82 (9.67, 10.1)	0.861
Child cotinine (ng/mL)	7.38 (2.00, 18.7)	9.15 (2.00, 29.9)	0.255
Child BMI	18.6 (16.3, 21.4)	18.4 (16.6, 21.7)	0.720
Maternal education, n (%)			0.066
Primary	34 (26.1)	29 (20.9)	
Secondary	47 (36.2)	38 (27.3)	
University	49 (37.7)	72 (51.8)	
Child somatic problems (t-scores)	55 (50, 61)	57 (50, 61)	0.549
Child thought problems (t-scores)	51 (50, 58)	51 (50, 58)	0.571
Child total behavior problems (t-scores)	51.5 (47, 58)	53 (49, 61)	0.517

Data are presented as medians and interquartile ranges (IQRs) for continuous variables or n (%) for categorical variables.

^a Value of hypothesis testing calculated using the Kruskal-Wallis test for continuous variables and the Chi-square test for categorical variables.

Table S2. Bisulfite-converted primers used for PCR amplification.

Gene	Forward primer	Reverse primer
<i>BDNF</i>	GATTTTGGTAATTAGTGTATTAGA GTGTT	/5Biosg/ATCAACCAAAAACCTCCATT TAATCTC

Table S3. Pearson correlations coefficients among BDNF biomarkers.

		Correlations								
		Serum BDNF protein levels	Urinary BDNF protein levels	CpG1	CpG2	CpG3	CpG4	CpG5	CpG6	CpG mean
Serum BDNF protein levels	Pearson Correlation	1	-.080	.111	-.203*	-.189	-.240*	-.161	-.091	-.178
	Sig. (2-tailed)		.389	.264	.040	.056	.015	.104	.359	.073
	N	120	119	103	103	103	103	103	103	103
Urinary BDNF protein levels	Pearson Correlation	-.080	1	.183	.006	-.043	-.010	-.076	-.066	-.004
	Sig. (2-tailed)	.389		.062	.950	.667	.918	.444	.506	.969
	N	119	121	104	104	104	104	104	104	104
CpG1	Pearson Correlation	.111	.183	1	.285**	.201*	-.211*	-.070	-.210*	.157
	Sig. (2-tailed)	.264	.062		.003	.038	.030	.474	.030	.106
	N	103	104	107	107	107	107	107	107	107
CpG2	Pearson Correlation	-.203*	.006	.285**	1	.624**	.359**	.487**	.331**	.636**
	Sig. (2-tailed)	.040	.950	.003		.000	.000	.000	.000	.000
	N	103	104	107	107	107	107	107	107	107
CpG3	Pearson Correlation	-.189	-.043	.201*	.624**	1	.608**	.708**	.654**	.851**
	Sig. (2-tailed)	.056	.667	.038	.000		.000	.000	.000	.000
	N	103	104	107	107	107	107	107	107	107
CpG4	Pearson Correlation	-.240*	-.010	-.211*	.359**	.608**	1	.807**	.814**	.848**
	Sig. (2-tailed)	.015	.918	.030	.000	.000		.000	.000	.000
	N	103	104	107	107	107	107	107	107	107
CpG5	Pearson Correlation	-.161	-.076	-.070	.487**	.708**	.807**	1	.868**	.911**
	Sig. (2-tailed)	.104	.444	.474	.000	.000	.000		.000	.000
	N	103	104	107	107	107	107	107	107	107
CpG6	Pearson Correlation	-.091	-.066	-.210*	.331**	.654**	.814**	.868**	1	.860**
	Sig. (2-tailed)	.359	.506	.030	.000	.000	.000	.000		.000
	N	103	104	107	107	107	107	107	107	107
CpG mean	Pearson Correlation	-.178	-.004	.157	.636**	.851**	.848**	.911**	.860**	1
	Sig. (2-tailed)	.073	.969	.106	.000	.000	.000	.000	.000	
	N	103	104	107	107	107	107	107	107	107

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Urinary BDNF protein concentrations were normalized by urinary creatinine levels.

Table S4. Distribution of CBCL behavior t-scores in INMA-Granada adolescent boys (n=130).

Behavioral functions				
Syndrome scores	Score range	Mean (SD)	Median (IQR)	N (%) borderline/clinical*
Anxious/Depressed	50-78	54.7 (5.8)	51 (50, 57)	30 (23.1)
Withdrawn	50-81	56.9 (6.2)	54 (53, 60)	43 (33.1)
Somatic complaints	50-79	58.0 (7.3)	58 (50, 61)	46 (35.4)
Social problems	50-75	54.6 (5.7)	51 (50, 58)	28 (21.5)
Thought problems	50-82	54.5 (5.7)	51 (50, 56)	17 (13.1)
Attention problems	50-75	55.4 (6.1)	53 (50, 59)	24 (18.5)
Rule-breaking problems	50-89	53.5 (5.5)	51 (50, 54)	17 (13.1)
Aggressive behavior	50-81	54.8 (5.5)	52 (50, 58)	27 (20.8)
Composite scores				
Internalizing problems	50-80	56.7 (6.7)	55 (51, 61)	39 (30.0)
Externalizing problems	50-69	53.8 (4.4)	51 (51, 57)	18 (13.8)
Total problems	50-80	57.1 (7.3)	50 (56, 60)	30 (23.1)

CBCL: Child behavior checklist (6–18 years). Higher t-scores mean more problems in all scales.

** T-scores categorized as borderline or clinical (≥ 60), or normal (< 60).*

Table S5. Mediation analysis showing associations between childhood urinary BPA concentrations, BDNF DNA methylation and thought/somatic problems t-scores during adolescence (n=103).

BDNF methylation marker	CBCL scale	Indirect effect β (95% CI)	Direct effect β (95% CI)	Total effect β (95% CI)	Estimated percent mediated (%)^a
Log-2 CpG5	Thought problems	0.13 (-0.04, 0.40)	0.55 (-0.33, 1.43)	0.68 (-0.19, 1.55)	19%
	Somatic Complaints	0.09 (-0.18, 0.45)	0.81 (-0.37, 1.99)	0.90 (-0.26, 2.06)	10%
Log-2 CpG6	Thought problems	0.23 (0.01, 0.57)	0.45 (-0.45, 1.34)	0.68 (-0.19, 1.55)	34%
	Somatic Complaints	0.17 (-0.22, 0.62)	0.72 (-0.48, 1.93)	0.90 (-0.26, 2.06)	19%
Log-2 CpG mean	Thought problems	0.09 (-0.08, 0.34)	0.60 (-0.30, 1.49)	0.68 (-0.19, 1.55)	13%
	Somatic Complaints	-0.00 (-0.34, 0.30)	0.90 (-0.29, 2.09)	0.90 (-0.26, 2.06)	0%

Continuous log₂-transformed BPA concentrations and CpG methylation percentage, and continuous thought problems t-scores were used. Models were adjusted for age and BMI z-scores (continuous) at neuropsychological evaluation (15-17 years), maternal education (primary, secondary or university), and urinary cotinine at 9-11y. (continuous).

^a *The direct effect, indirect effect, and total effect reflect the natural beta coefficients (β) and 95% Confidence Intervals (95% CI). The indirect effect represents the mediated effect.*

^b *Percent mediated = indirect effect / (direct effect + indirect effect) \times 100.*