

Supplementary Material

Attention, cognitive control and motivation in ADHD: Linking event-related brain potentials and DNA methylation patterns in boys at early school age

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Table S-1: Performance and event-related potential (ERP) measures for the three groups of boys classified along their FBB-ADHS total score: Controls (≤ 0.5); ADHD-low: ($> 0.5 / \leq 1$); ADHD-high (> 1). Mean values \pm standard deviations for those measures being associated with the FBB-ADHS total score are presented. Comparing Controls vs. ADHD-high using t-tests revealed significant results for all measures. Abbreviations: w/o = without; dRTVar = difference of reaction time variability between blocks with and without motivational incentives.

	Controls (N = 40)		ADHD-low (N = 29)		ADHD-high (N = 13)	
	w/o incentives	incentives	w/o incentives	incentives	w/o incentives	incentives
Reaction time variability (ms)	117.3 \pm 48.9	104.4 \pm 34.4	114.8 \pm 50.3	100.8 \pm 32.2	150.4 \pm 58.9	107.1 \pm 38.4
Cue-P3 (Pz, μ V)	10.3 \pm 3.6	10.9 \pm 3.6	8.7 \pm 3.7	8.9 \pm 4.0	7.4 \pm 3.6	8.2 \pm 3.4
Nogo-P3						
CPz (μ V)	16.8 \pm 5.8	16.8 \pm 4.4	15.5 \pm 6.1	15.2 \pm 5.3	12.1 \pm 3.6	12.3 \pm 5.7
Pz (μ V)	15.1 \pm 5.9	15.6 \pm 4.5	14.6 \pm 5.8	14.5 \pm 5.9	11.1 \pm 4.5	12.3 \pm 6.4

Performance and ERP measures	Comparisons of Controls and ADHD-high (t-tests)	
dRTVar (ms)	t(51) = -2.29	p = 0.026
Cue-P3 (Pz)	t(51) = 2.74	p = 0.008
Nogo-P3 (CPz)	t(51) = 3.21	p = 0.002

Table S-3: Mean DNA methylation \pm SD (residuals of the preprocessing regression analysis) for the three groups of boys classified along their FBB-ADHS total score: Controls (≤ 0.5); ADHD-low: ($> 0.5 / \leq 1$); ADHD-high (> 1). Only CpG sites are included that fulfilled our statistical criteria (inter alia significant associations with the behavioural and functional level). Comparing Controls vs. ADHD-high using t-tests revealed significant results for all CpGs.

Gene – CpG	Controls (N=31)	ADHD-low (N=25)	ADHD-high (N=11)
<i>COMT</i> - cg08289189	-0.0295 \pm 0.0652	0.0145 \pm 0.0857	0.0566 \pm 0.0611
<i>ANKK1</i> - cg15946653	-0.0074 \pm 0.0240	0.0047 \pm 0.0195	0.0109 \pm 0.0282
<i>BDNF</i> - cg11806762	0.0250 \pm 0.0450	-0.0016 \pm 0.0621	-0.0276 \pm 0.0534
<i>NGFR</i> - cg04613258	-0.0149 \pm 0.0251	0.0070 \pm 0.0296	0.0183 \pm 0.0287
<i>DPP10</i> - cg19651219	-0.0176 \pm 0.0412	0.0065 \pm 0.0582	0.0328 \pm 0.0698
<i>TPH2</i> - cg14791008	-0.0221 \pm 0.0608	0.0097 \pm 0.0628	0.0507 \pm 0.0570

Gene – CpG	Comparisons of Controls and ADHD-high (t-tests)	
<i>COMT</i> - cg08289189	t(36) = -3.64	p = 0.0008
<i>ANKK1</i> - cg15946653	t(40) = -2.08	p = 0.0436
<i>BDNF</i> - cg11806762	t(40) = 3.17	p = 0.0029
<i>NGFR</i> - cg04613258	t(40) = -3.64	p = 0.0008
<i>DPP10</i> - cg19651219	t(40) = -2.88	p = 0.0064
<i>TPH2</i> - cg14791008	t(39) = -3.45	p = 0.0013

Table S-4: Additional analyses taking prenatal risk factors (maternal smoking, alcohol exposure, maternal depressive symptomatology) and medication into account. For the linear regression models, coefficients \pm standard errors are presented.

Linear regression analyses - prenatal risk factors as covariates

Gene – CpG	Associations with ADHD total score	Nogo-P3
<i>COMT</i> - cg08289189	0.0401 \pm 0.0139, p = 0.0053	-0.0065 \pm 0.0020, p = 0.0024
<i>ANKK1</i> - cg15946653	0.0213 \pm 0.0058, p = 0.0005	-0.0017 \pm 0.0006, p = 0.0072
<i>BDNF</i> - cg11806762	-0.0287 \pm 0.0134, p = 0.0361	0.0043 \pm 0.0013, p = 0.0022
<i>NGFR</i> - cg04613258	0.0204 \pm 0.0077, p = 0.0098	-0.0019 \pm 0.0008, p = 0.0179
		Cue-P3
<i>DPP10</i> - cg19651219	0.0401 \pm 0.0139, p = 0.0053	-0.0057 \pm 0.0018, p = 0.0019
		dRTVar
<i>TPH2</i> - cg14791008	0.0550 \pm 0.0156, p = 0.0008	-4.8e-04 \pm 1.6e-04, p = 0.0045

Correlation analyses - excluding participants receiving methylphenidate (MPH, N = 4)

Gene - CpG	Associations with ADHD total score	Nogo-P3
<i>COMT</i> - cg08289189	r = 0.365, p = 0.0045	r = -0.341, p = 0.0083
<i>ANKK1</i> - cg15946653	r = 0.367, p = 0.0031	r = -0.306, p = 0.0147
<i>BDNF</i> - cg11806762	r = -0.202, p = 0.1125	r = 0.376, p = 0.0024
<i>NGFR</i> - cg04613258	r = 0.290, p = 0.0213	r = -0.28134, p = 0.0256
		Cue-P3
<i>DPP10</i> - cg19651219	r = 0.367, p = 0.0031	r = -0.361, p = 0.0037
		dRTVar
<i>TPH2</i> - cg14791008	r = 0.405, p = 0.0012	r = -0.378, p = 0.0027

Figure S-1: Scatter plots (with regression lines) showing associations between DNA methylation and the FBB-ADHS total score. 5 of the 6 CpGs were hypermethylated in boys with higher ADHD scores. It has to be noted that DNA methylation is depicted as residuals which are centered at 0. FBB-ADHS: German ADHD rating scale.

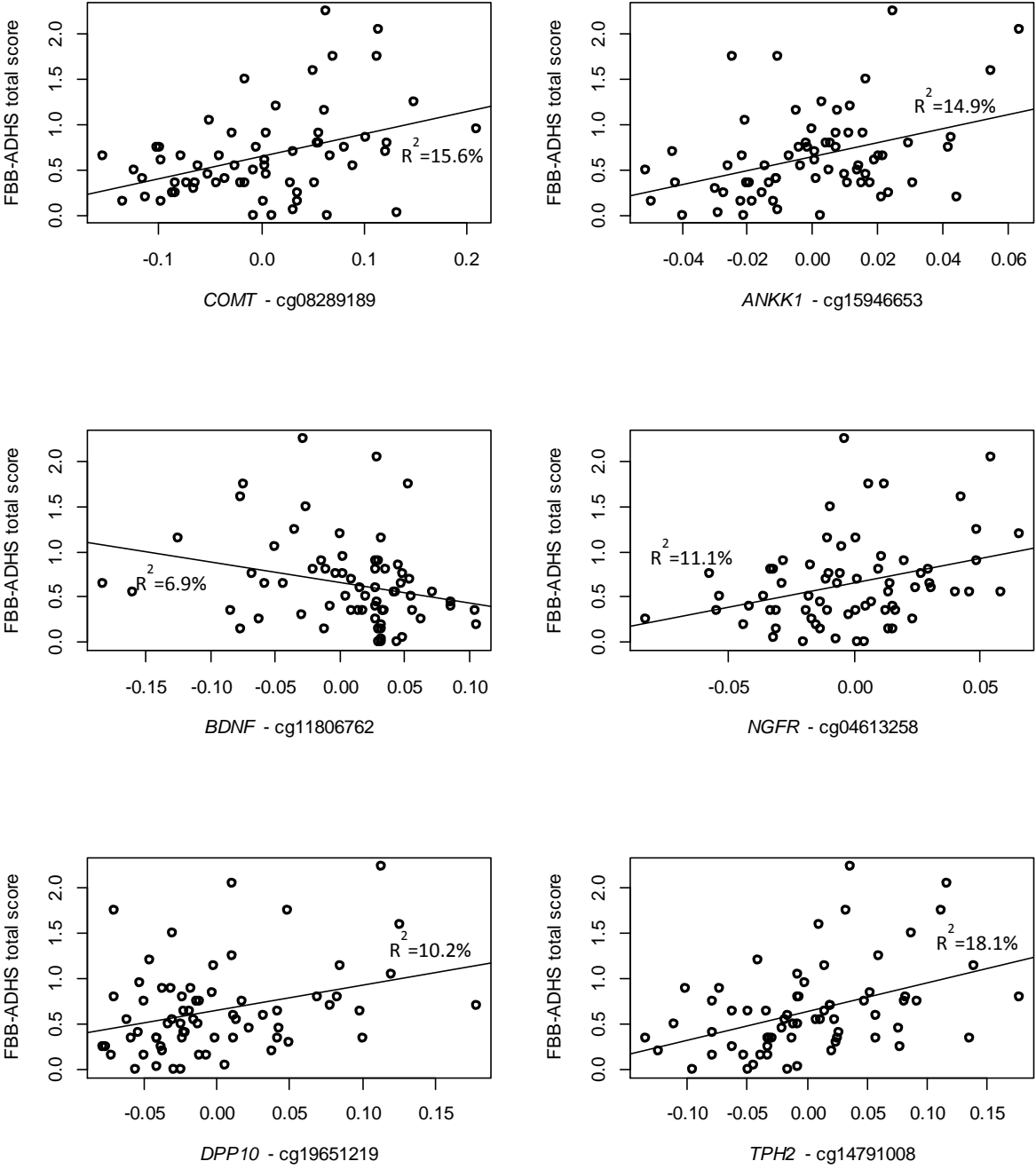


Table S-5: Candidate genes and number of CpGs

60 candidate genes (comprising 2031 CpGs) were included in the analysis. All CpGs linked to those candidate genes as defined in the HumanMethylation450K Manifest File (v.1.2) except probes containing SNPs and virtually showing no variance (i.e., mean beta values below 0.01 or above 0.99) were considered.

Genes	no. CpGs	Genes	no. CpGs
<i>DRD1</i>	8	<i>NTRK2</i>	14
<i>DRD2</i>	16	<i>NTRK3</i>	30
<i>DRD3</i>	9	<i>CDH13</i>	51
<i>DRD4</i>	17	<i>GFOD1</i>	38
<i>DRD5</i>	12	<i>MTA3</i>	13
<i>DAT1/SLC6A3</i>	45	<i>SPATA13</i>	42
<i>COMT</i>	29	<i>UNC5B</i>	26
<i>ANKK1</i>	10	<i>ASTN2</i>	24
<i>DDC</i>	34	<i>CSMD2</i>	68
<i>DBH</i>	8	<i>ITGAE</i>	32
<i>NET1/SLC6A2</i>	16	<i>ITGA11</i>	28
<i>ADRA2A</i>	11	<i>CDH23</i>	98
<i>ADRA2C</i>	9	<i>GPC6</i>	63
<i>ADRA1A</i>	17	<i>CTNNA2</i>	76
<i>ADRA1B</i>	17	<i>NAV2</i>	114
<i>ADRB1</i>	7	<i>KCNIP4</i>	45
<i>ADRB2</i>	8	<i>KCNIP1</i>	35
<i>SLC6A4</i>	15	<i>DPP10</i>	45
<i>HTR1B</i>	10	<i>FHIT</i>	55
<i>HTR2A</i>	21	<i>KCNC1</i>	26
<i>TPH2</i>	19	<i>SNAP25</i>	16
<i>BDNF</i>	50	<i>CHRNA4</i>	18
<i>NGF</i>	23	<i>SLC9A9</i>	20
<i>NGFR</i>	24	<i>SUCNR1</i>	21
<i>NTF3</i>	36	<i>NOS1</i>	20
<i>NTF4</i>	6	<i>GRIN1</i>	31
<i>CNTF</i>	23	<i>GRIN2A, GRIN2B, GRIN2C, GRIN2D</i>	134
<i>CNTFR</i>	12	<i>GRIN3A, GRIN3B</i>	25
<i>GDNF</i>	39	<i>MYT1L</i>	155
<i>NTRK1</i>	46	<i>VIPR2</i>	71

Table S-6: Sample characteristics for the three groups of boys classified along their FBB-ADHS total score: controls (≤ 0.5); ADHD-low: ($> 0.5 / \leq 1$); ADHD-high (> 1). The groups did not differ significantly regarding age, IQ, socioeconomic status and absence or presence of prenatal maternal smoking and prenatal maternal depressive symptomatology. There was a tendency regarding prenatal alcohol exposure (higher probability in the ADHD-high group).

	Controls	ADHD-low	ADHD-high	Statistics
N	40	29	13	
Age (years)	7.58 \pm 0.52	7.52 \pm 0.68	7.45 \pm 0.43	F(2,79) = 0.24, n.s.
IQ	105.4 \pm 8.1	104.5 \pm 11.6	101.3 \pm 10.7	F(2,79) = 0.87, n.s.
Socioeconomic status	11.6 \pm 1.8	11.5 \pm 2.0	10.5 \pm 2.3	F(2,79) = 1.59, n.s.
ADHD rating scale - total score	0.29 \pm 0.14	0.72 \pm 0.12	1.55 \pm 0.39	F(2,79) = 207.9, p<0.001
Prenatal risk factors:				
Maternal smoking	3 (7.5%)	7 (24.1%)	2 (15.4%)	$\chi^2 = 3.73$, p = n.s.
Alcohol exposure	7 (17.5%)	5 (17.2%)	6 (46.2%)	$\chi^2 = 5.28$, p = 0.07
Maternal depressive symptoms	5 (12.5%)	4 (13.8%)	3 (23.1%)	$\chi^2 = 0.90$, n.s.