1 **DNA methylation and general psychopathology in childhood:**

2 **An epigenome-wide meta-analysis from the PACE consortium**

3 Jolien Rijlaarsdam[1]*, Marta Cosin-Tomas[2,3,4]*#, Laura Schellhas[5,6]*, Sarina Abrishamcar[7],
4 Anni Malmberg[8], Alexander Neumann[9], Janine F. Felix[10,11], Jordi Sunyer[2,3,4], Kristine B.
5 Gutzkow[12], Regina Grazuleviciene[13], John Wright[14], Mariza Kampouri[15], Heather J. Zar[16,17],
6 Dan J. Stein[18,19], Kati Heinonen[20,8], Katri Räikkönen[8], Jari Lahti[8], Anke Huels[7,21], Doretta
7 Caramaschi[22,23]**, Silvia Alemany[2,24,25]**, Charlotte A. M. Cecil[1,26,27]**#

8 [1] Department of Child and Adolescent Psychiatry/ Psychology, Erasmus MC University Medical
9 Center Rotterdam, Rotterdam, the Netherlands
10 [2] ISGlobal, Barcelona Institute for Global Health, Barcelona, Spain
11 [3] Universitat Pompeu Fabra, Barcelona, Spain
12 [4] Centro de investigación biomédica en red en epidemiología y salud pública (ciberesp), Madrid, Spain
13 [5] School of Psychological Science, MRC Integrative Epidemiology Unit, University of Bristol, Bristol,
14 UK
15 [6] Institute for Sex Research, Sexual Medicine and Forensic Psychiatry, University Medical Center
16 Hamburg-Eppendorf, Germany
17 [7] Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA
18 [8] Department of Psychology & Logopedics, University of Helsinki, Finland
19 [9] VIB Center for Molecular Neurology, Antwerp, Belgium
20 [10] The Generation R Study Group, Erasmus MC University Medical Center Rotterdam, Rotterdam, the
21 Netherlands
22 [11] Department of Pediatrics, Erasmus MC University Medical Center Rotterdam, Rotterdam, the
23 Netherlands
24 [12] Department of Environmental Health, Norwegian Institute of Public Health (NIPH), Oslo, Norway
25 [13] Department of Environmental Science, Vytautas Magnus University, 44248 Kaunas, Lithuania
26 [14] Bradford Institute for Health Research, Bradford Teaching Hospitals NHS Foundation Trust,
27 Bradford, UK
28 [15] Department of Social Medicine, University of Crete, Greece
29 [16] Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital, University
30 of Cape Town, SA
31 [17] South African Medical Research Council (SAMRC) Unit on Child and Adolescent Health, University
32 of Cape Town, Cape Town, South Africa
33 [18] Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa
34 [19] South African Medical Research Council (SAMRC) Unit on Risk and Resilience in Mental Disorders,
35 Neuroscience Institute, University of Cape Town, Cape Town, South Africa
36 [20] Psychology/ Welfare Sciences, Faculty of Social Sciences, Tampere University, Finland
37 [21] Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University,
38 Atlanta, GA, USA
39 [22] Medical Research Council Integrative Epidemiology Unit, Population Health Science, Bristol Medical
40 School, University of Bristol, Bristol, United Kingdom
41 [23] Department of Psychology, University of Exeter, Exeter, United Kingdom
42 [24] Psychiatric Genetics Unit, Group of Psychiatry, Mental Health and Addiction, Vall d'Hebron
43 Research Institute (VHIR), Universitat Autònoma de Barcelona, Barcelona, Spain

44 [25] Biomedical Network Research Centre on Mental Health (CIBERSAM), Instituto de Salud Carlos III,
45 Barcelona, Spain
46 [26] Department of Epidemiology, Erasmus MC University Medical Center Rotterdam, Rotterdam, the
47 Netherlands
48 [27] Molecular Epidemiology, Department of Biomedical Data Sciences, Leiden University Medical
49 Center, Leiden, The Netherlands
50
51 */** Contributed equally
52 # Co-corresponding authors:
53 Charlotte A. M. Cecil
54 c.cecil@erasmusmc.nl
55 Marta Cosin-Tomas
56 marta.cosin@isglobal.org

Abstract

The general psychopathology factor (GPF) has been proposed as a way to capture variance shared between psychiatric symptoms. Despite a growing body of evidence showing both genetic and environmental influences on GPF, the biological mechanisms underlying these influences remain unclear. In the current study, we conducted epigenome-wide meta-analyses to identify both probe- and region-level associations of DNA methylation (DNAm) with school-age general psychopathology in six cohorts from the Pregnancy And Childhood Epigenetics (PACE) Consortium. DNAm was examined both at birth (cord blood; prospective analysis) and during school-age (peripheral whole blood; cross-sectional analysis) in total samples of N=2,178 and N=2,190, respectively. At school-age, we identified one probe (cg11945228) located in the Bromodomain-containing protein 2 gene (*BRD2*) that negatively associated with GPF ($p=8.58\times10^{-8}$). We also identified a significant differentially methylated region (DMR) at school-age ($p=1.63\times10^{-8}$), implicating the SHC Adaptor Protein 4 (*SHC4*) gene and the EP300-interacting inhibitor of differentiation 1 (*EID1*) gene that have been previously implicated in multiple types of psychiatric disorders in adulthood, including obsessive compulsive disorder, schizophrenia, and major depressive disorder. In contrast, no prospective associations were identified with DNAm at birth. Taken together, results of this study revealed some evidence of an association between DNAm at school-age and GPF. Future research with larger samples is needed to further assess DNAm variation associated with GPF.

78 **Introduction**

79 Psychiatric disorders or symptoms co-occur more often than would be expected by chance
80 alone.[1,2] In light of the negative clinical and functional outcomes associated with psychiatric
81 co-occurrence,[3,4] it is important to identify early indicators of risk and underlying biological
82 mechanisms. There is accumulating evidence that, as early as in childhood, the shared
83 variance between psychiatric disorders or symptoms may be usefully represented by a general
84 psychopathology factor (GPF).[5–8] This GPF in childhood has been found to show temporal
85 stability[6] and to predict long-term functional and psychiatric outcomes in adolescence
86 throughout adulthood.[9,10] Although previous research has found evidence for both genetic and
87 environmental influences on GPF,[7,11–16] the biological mechanisms underlying these
88 influences remain unclear.

89 One of the ways by which genetic and environmental factors might contribute to
90 disease susceptibility is through epigenetic mechanisms that regulate gene expression, such as
91 DNA methylation (DNAm).[17] Studies have shown that variation in DNAm is influenced by a
92 dynamic interplay of genetic and environmental factors.[18] In turn, alterations in DNAm
93 patterns across the genome in peripheral tissues including cord blood, and peripheral blood
94 have been found to associate with a wide range of child and adult mental health outcomes,
95 such as conduct problems, attention deficit hyperactivity disorder (ADHD) symptoms, major
96 depressive disorder (MDD), and schizophrenia.[19–22] Despite a growing body of research
97 implicating an involvement of DNAm in individual mental health outcomes, much less work
98 has focused on the relationship between DNAm and general psychopathology.[23] To the best
99 of our knowledge, only one study examined the association between genome-wide DNAm
100 patterns and GPF in childhood. In this study, data were analyzed cross-sectionally in one
101 cohort, focusing on wider biological networks (so called ‘modules’) of co-methylated DNAm
102 probes across the genome.[23] As such, we still lack knowledge on how GPF relates to single

103 DNAm probes and the extent to which associations vary across time (i.e., both cross-sectional
104 and prospective associations). Large multi-cohort epigenome-wide studies, which allow for
105 increased power and generalizability, are needed to improve our understanding of the
106 biological mechanisms underlying shared variance across mental health problems.

107 We conducted epigenome-wide meta-analyses to investigate both probe-level and
108 region-based associations of DNAm with school-age GPF in the Pregnancy And Childhood
109 Epigenetics (PACE) Consortium. Because it is unclear at which time point differential DNAm
110 may be most relevant to GPF, we examined DNAm both at birth (cord blood; prospective
111 study; pre-symptom manifestation) and at school-age (peripheral whole blood; cross-sectional
112 study) in pooled samples of N=2,178 and N=2,190 children, respectively.

113 **Methods**

114 **Participants**

115 The prospective analyses included four cohorts from PACE, using complete data on DNAm at
116 birth, general psychopathology in childhood and covariates: the Avon Longitudinal Study of
117 Parents and Children (ALSPAC), Drakenstein Child Health Study (DCHS), Generation R
118 (GENR), and INfancia y Medio Ambiente (INMA). These cohorts have a combined sample
119 size of 2,178 (see Table 1). All prospective cohorts included participants of European
120 ancestry, except for DCHS, which included participants of predominantly Black African
121 ancestry or mixed ancestry. See Supplementary Methods for full cohort descriptions.

122 The cross-sectional analyses included four cohorts from the PACE consortium, using
123 complete data on DNAm and general psychopathology in childhood, as well as covariates;
124 ALSPAC, GENR, Glycyrrhizin in Licorice (GLAKU), and Human Early Life Exposome
125 (HELIX; including six jointly analyzed sub cohorts). These cohorts have a combined sample
126 size of 2,190 (see Table 1). All cross-sectional cohorts included participants of European

127 ancestry, except for HELIX, which included participants of European ancestry and
128 participants with a Pakistani background living in the United Kingdom, which were analyzed
129 as separate cohorts in our meta-analysis.

130 **Measures**

131 *DNA methylation*

132 DNAm was assessed with the Illumina Infinium HumanMethylation450 (ALSPAC, DCHS,
133 GENR, HELIX, INMA) or the Infinium HumanMethylationEPIC (DCHS, GLAKU)
134 BeadChip assays in cord blood and in peripheral whole blood at ages 7-12 years. The cohorts
135 performed sample processing, quality control (QC) and normalization based on their preferred
136 protocols as described in the Supplementary methods. We used normalized, untransformed
137 beta values, ranging from 0 (fully unmethylated) to 1 (fully methylated). Methylation levels
138 that fell outside of the lower quartile minus 3 × interquartile or upper quartile plus 3 ×
139 interquartile range were removed.

140 We excluded probes with a call rate <90%, control probes, and probes that mapped to
141 X/Y chromosomes. Following Zhou et al.,[24] we further excluded probes with poor base
142 pairing quality (lower than 40 on 0-60 scale), probes with non-unique 30bp 3'-subsequence
143 (with cross-hybridizing problems), Infinium II probes with SNPs of global MAF over 1%
144 affecting the extension base, and probes with a SNP in the extension base that causes a color
145 channel switch from the official annotation. We also excluded a subset of probes (n=69) that
146 have shown to be unreliable in a recent comparison of the Illumina 450K and EPIC
147 BeadChips.[25] At the meta-analysis level, we excluded probes which were available in <50%
148 of the cohorts and <50% of the participants. After QC, 404,017 probes remained at birth and
149 413,497 probes remained at school-age.

150 *General psychopathology factor*

151 Mental health symptoms were assessed when children were aged **6-12 years**, depending on
152 the cohort. Parent-rated instruments were used, including (i) the Child Behavior Checklist 6-
153 18 (CBCL/6-18) in DCHS, GENR, GLAKU, HELIX, and INMA, and (ii) the Development
154 and Well-being Assessment (DAWBA) in ALSPAC. Instruments are described in more
155 detailed in the Supplementary methods. Whereas a single general factor loaded on all CBCL
156 or DAWBA problem subscales, two specific factors loaded on internalizing (CBCL:
157 anxious/depressed, withdrawn/depressed, somatic complaints; DAWBA: generalized anxiety
158 disorder, major depressive disorder, social phobia, separation anxiety, specific phobia) versus
159 externalizing (CBCL: rule-breaking behavior, aggressive behavior, attention problems;
160 DAWBA: attention deficit hyperactivity disorder, oppositional defiant disorder, conduct
161 disorder) subscales. For the CBCL, three subscales (social problems, thought problems, other
162 problems) were indicators of the general factor but were not part of the specific internalizing
163 or externalizing factors. Of note, GLAKU included only two of these three CBCL subscales
164 as the 'other problems' subscale was not available.

165 The internalizing and externalizing factors were allowed to correlate with each other but not
166 with the general factor. As such, the general factor represents the shared variance among all
167 problem scales that is independent of the more specific internalizing and externalizing factors.
168 **Previous research reported negative correlations between the GPF and cognitive**
169 **outcomes** [10,14,26]**. To support the criterion validity of the GPF, we estimated the**
170 **correlation between the GPF and child cognition across the cohorts.**

171 *Covariates*

172 We adjusted for the following potential confounders: child sex, gestational age at birth, child
173 age at the assessment of outcome, maternal age, maternal educational level, prenatal maternal
174 smoking status, cell-type proportions estimated using standard algorithms for DNAm at

175 birth[27] or childhood,[28] ancestry (depending on the specific cohort), and technical covariates
176 (e.g., batch) (see **Supplementary methods**). **To test the robustness of findings when using**
177 **a different method to estimate cell-type proportions, we re-ran the cross-sectional**
178 **EWAS analyses within the cohort with the largest sample size (HELIX, itself comprised**
179 **of different participating cohorts) with newly estimated cell proportions using IDOL**
180 **(Salas et al., 2018 [29]) instead of Houseman's approach[28].**

181

182 **Statistical analyses**

183 *General psychopathology factor*

184 We used confirmatory factor analysis (CFA) to fit a general psychopathology model in the
185 full samples with mental health data available (see **Supplementary Information 1**). Each
186 cohort ran the CFA according to a predefined script, using the Lavaan statistical package[32] in
187 R (https://www.r-project.org/). GPF scores were extracted, winsorized at +/- 3SD, and
188 standardized.

189 *Cohort-specific EWAS*

190 Each cohort ran the EWAS according to a predefined analysis plan, using robust linear
191 regression (rlm; *MASS* R-package) to account for potential heteroscedasticity and non-
192 normality. Cohorts excluded all multiple births and chose one random sibling per non-twin
193 sibling pair.

194 *Meta-analysis*

195 The cohort-specific results were meta-analyzed at Erasmus MC Rotterdam. A shadow meta-
196 analysis was conducted independently at the Barcelona Institute for Global Health. We

197 performed an inverse-variance weighted fixed effects approach using R and METAL.[33]
198 Probes were annotated using *meffil*.[34] Genome-wide significance was defined at the
199 Bonferroni-corrected threshold of $p < 1\times10^{-7}$, and suggestive significance at $p < 1\times10^{-5}$. We
200 included $p < 1\times10^{-4}$ specifically for pathway and enrichment analyses to allow a sufficient
201 number of genes to be included.

202 We ran two sensitivity meta-analyses. First, we included only cohorts of
203 predominantly European ancestry to check if the results of the main analysis were influenced
204 by ancestry. Second, we performed leave-one-out meta-analyses for hits showing genome-
205 wide significant associations with GPF to ensure that associations were not driven by a single
206 cohort.

207 *Differentially methylated regions*

208 Differentially methylated regions (DMRs) were identified using the dmrff package[35] in R.
209 This method first identifies candidate DMRs by screening the meta-level EWAS results for
210 genomic regions each covered by a sequence of CpG sites with EWAS effects in the same
211 direction, EWAS *p*-values <0.05, and <500bp gaps between consecutive CpG sites. Then,
212 summary statistics are calculated for each candidate DMR within each of the cohorts by meta-
213 analyzing the cohort-level EWAS summary statistics of the CpG sites in the region. Meta-
214 analysis is performed by a variation of inverse weighted fixed effects meta-analysis that
215 accounts for non-independence between CpG sites. Finally, for each candidate DMR, the
216 summary statistics from each cohort are meta-analyzed to obtain a cross-cohort meta-analyzed
217 DMR statistic and *p*-value.

218 *Follow-up analyses*

219 Individual probes showing genome-wide or suggestive significance were looked up in
220 the EWAS catalog[36] and EWAS atlas[37] to examine potential associations with exposures and

221 health outcomes based on existing studies. To further characterize potential environmental -
222 as well as genetic – influences on these sites, we used two different tools: 1) a heritability tool
223 quantifying additive genetic influences as opposed to shared and non-shared environmental
224 influences on DNAm, based on data from monozygotic and dizygotic twins[38]; and 2) the
225 GoDMC database (http://mqtldb.godmc.org.uk/) as a more specific tool for identifying
226 genetic influences on DNAm levels via mQTL mapping. GoDMC is a large-scale
227 collaborative effort including 36 cohorts (4 of which participated in this study: INMA,
228 ALSPAC, GENR, GLAKU), based on whole blood from over 27,000 European samples. We
229 characterized cross-tissue correspondence of DNAm using the Blood Brain DNA Methylation
230 Comparison Tool by Hannon et al.[39], the Blood–Brain Epigenetic Concordance (BECon)[40],
231 and the Iowa Methylation Array Graphing for Experimental Comparison of Peripheral tissue
232 & Gray matter (IMAGE-CpG)[41]. To assess whether methylation levels of CpGs were
233 associated with the expression levels of nearby genes in child blood, we consulted the HELIX
234 Expression Quantitative Trait Methylation (eQTM) catalogue
235 (https://helixomics.isglobal.org/), generated from samples overlapping with those included in
236 this study (from the HELIX cohort). Finally, chromatin states associated to the most
237 significant CpGs were assessed using ROADMAP blood 15 reference chromatin states
238 (annotation and enrichment analysis conducted using the Enrichment module of the EASIER
239 R package). Genome Browser (UCSC) was used to further explore the genomic context of the
240 identified DMR.

241 To identify broader pathways and enrichment for molecular functions, we used the
242 gene ontology (GO-biological processes, GO-molecular functions and GO-cellular
243 components), the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, and the
244 Molecular Signature Database (MSigDB) enrichment methods from the missMethyl R
245 package,[42] as implemented in the Functional Enrichment module of the EASIER R package.[43]

246 We ran GWAS enrichment analyses for EWAS using the GenomicRanges Package,[44] to
247 identify genomic regions of EWAS suggestive hits ($p< 1\times10^{-4}$) that overlapped with the 378
248 genome-wide significant loci previously reported in GWASs on general psychopathology,[16]
249 schizophrenia,[45] neuroticism[46], ADHD[47] or anxiety[48] (0.5Mb window centered to the genomic
250 locus indicated in the original studies).

251 **Results**

252 **General psychopathology factor**

253 All mental health subscales had significant loadings on the general factor across all cohorts,
254 with all loadings >0.30. For full details on the GPF loadings, correlations, and model fit, see
255 Supplementary Table 1. The loadings of the mental health subscales on the specific
256 internalizing and externalizing factors tended to be lower and were less consistent across the
257 cohorts, as were the correlations between these specific factors. In INMA and HELIX, a
258 model including the correlation between the specific internalizing and externalizing factors
259 did not fit the data well (see Supplementary Information 1). Therefore, in both INMA and
260 HELIX, the specific internalizing and externalizing factors were not allowed to correlate (i.e.,
261 completely orthogonal model; see Supplementary Table 1). In line with previous research,[5,7,14]
262 GPF consistently negatively correlated with child cognition (see Supplementary methods)
263 across the cohorts (mean r=-0.12, range=-0.08 to -0.13).

264 **Epigenome-wide meta-analysis**

265 Descriptive statistics across the cohorts are shown in Supplementary Table 2. We note that
266 some differences were observed in GPF levels and sociodemographic characteristics between
267 the full cohort samples and analytical subsamples used in the present study (see
268 Supplementary Table 2). These differences varied depending on the specific cohort and
269 variable examined. We prospectively examined associations of DNAm at birth (n=2,178) at

404,017 CpG sites with GPF at school-age. There was no evidence of genomic inflation in the cohort-specific EWASs (range λ=0.95-1.14), nor in the meta-analysis (λ=1.08, see also Figure 1). As can be seen in Figure 2, no CpG reached genome-wide significance at $p < 1\times10^{-7}$, with four CpGs showing $p < 1\times10^{-5}$ (see Table 2). For the top hit (cg02084087), annotated to *TNFRSF25* (TNF Receptor Superfamily Member 25), a 10-point increase in percentage methylation was related to a 0.43 SD increase in general psychopathology symptoms (p=5.54$\times10^{-6}$).

In the cross-sectional meta-analysis of DNAm at school-age (n=2,190) at 413,497 sites, one CpG reached genome-wide significance (see Figure 2). For this CpG probe (cg11945228), mapped to *BRD2* (Bromodomain-containing protein 2 gene), a 10-point increase in percentage methylation was related to a 3.70 SD decrease in general psychopathology symptoms (p=8.58$\times10^{-8}$). Of note, there was a negative association between DNA methylation at this CpG and GPF in all cohorts except for the HELIX-Pakistani cohort. It is not possible based on the present data however to establish whether this may reflect an ancestry-specific association pattern or the influence of other cohort-specific factors (Supplementary Figure 2). Twenty other CpGs showed $p < 1\times10^{-5}$. These 21 top hits identified at school-age did not overlap with the ones observed at birth. Furthermore, as shown in Supplementary Table 3, the significant hit identified at school-age did not reach nominal significance (p <0.05) at birth (B=5.28, SE=3.76, p=0.16). Nominally significant probes identified in childhood correlated at r=0.004, p=0.55 (n=23,764) with respective probes at birth.

Sensitivity analyses

Restricting the meta-analysis to children with European ancestry did not change the overall pattern of results for both prospective (n=2,027) and cross-sectional (n=2,125) studies,

294 as evidenced by cross-meta-analysis correlations of effect estimates ($r_{prospective}$=0.99, $r_{cross-sectional}$=0.99) and consistent directions (95% and 96%, respectively) of effect estimates. The
295
296 top hit identified at school-age remained genome-wide significant (B=-38.02, SE=6.95,
297 p=4.47x10^{-8}).

298 Leave-one-out meta-analyses showed that the significant top hit identified during
299 childhood (cg11945228) was robust to excluding all individual cohorts, except GENR (for a
300 leave-one-out plot, see Supplementary Figure 1). Furthermore, when looking at the cohort-
301 level EWAS results, the cross-sectional association between cg11945228 and GPF was
302 statistically significant in GENR (B=-41.87, SE=7.78, p=7.39x10^{-8}) but not in the other
303 cohorts (all p >0.25, see Supplementary Figure 2 for a forest plot).

304 **Finally, we re-ran the cross-sectional EWAS analyses within HELIX using a**
305 **different method to estimate cell-type proportions (i.e. based on Salas et al., 2018[29]**
306 **instead of Houseman et al., 2012[28]. We found that the correlation between the**
307 **regression beta coefficients for all CpGs was very high (r = 0.97) (Supplementary Figure**
308 **3), indicating that results are highly concordant when using these two different methods.**

309 **Differentially methylated regions**

310 In the prospective analyses, there was no evidence of DMRs at birth associated with GPF. In
311 the cross-sectional analyses, one DMR at childhood was associated with GPF
312 (estimate=10166.54, SE=1800.19, p=1.63×10^{-8}). As shown in Supplementary Table 4, this
313 DMR included 6 CpGs mapped to the gene body of the SHC Adaptor Protein 4 gene (*SHC4*)
314 and close to the Transcription Start Site of the EP300 Interacting Inhibitor Of Differentiation
315 1 gene (*EID1*) at chromosome 15. From the 6 CpGs, 2 showed positive associations and 4
316 showed negative associations between methylation level and GPF.

317 **Follow-up analyses**

318 All probes showing significant or suggestive associations with DNAm had twin heritability
319 estimates available, showing mean additive genetic influences of r_{birth}=0.16 and
320 $r_{childhood}$=$9.44 x 10^{-2}$ (see Supplementary Table 5).

321 Of the four suggestive probes identified at birth, three were associated with at least one
322 known methylation quantitative trait locus (mQTL) (see Supplementary Table 5a) and one
323 (cg09437808) showed a concordant DNAm pattern (r >0.28, p <0.01) between blood and
324 several brain regions (the prefrontal cortex, entorhinal cortex, and the superior temporal
325 gyrus) according to Hannon et al. tool (see Supplementary Table 6a). This positive correlation
326 between blood and brain is also reported by IMAGE CPG tool (r = 0.35, p=0.31)
327 (Supplementary Table 7a) but not identified in BECon.

328 The genome-wide significant probe identified during childhood (cg11945228) was
329 unrelated to known mQTLs and showed non-significant correlations between blood and brain
330 DNAm (data only available in one of the three online tools used to assess this concordance)
331 (Supplementary Tables 5b and 6b). Of the 20 suggestive probes identified in childhood, ten
332 were associated with at least one known mQTL (see Supplementary Table 5b) and four
333 (cg22691524, cg09040034, cg25182716, cg18436008) showed a significant correlation
334 between blood and at least one brain region DNAm (r > 0.25, p <0.04; see Supplementary
335 Table 6b) according to Hannon et al tool. These sites also showed positive correlations in the
336 BECon (3 out of 4 CpGs; Supplementary Table 8b) and IMAGE CPG tool (3 of the 4 CpGs;
337 Supplementary Table 7b). None of the suggestive probes identified at birth or childhood
338 showed links to an eQTM. According to EWAS Atlas and EWAS Catalogue, methylation
339 levels at these top CpGs seem to be variable and sensitive to age, sex, tissue, or substance
340 exposure (smoking, alcohol, polychlorinated biphenyls), and/or associated to several traits
341 such as inflammatory and neurological diseases (rheumathoid arthritis, Behcet's disease,
342 myalgic encephalomyelitis, multiple sclerosis, among others) (see Supplementary Table 9).

343 The six probes in the DMR within the childhood analyses showed low evidence of
344 genetic effects, as indicated by both twin-based estimates (mean variance explained by
345 additive genetic influences r=0.007) and the lack of associations with known mQTLs. One
346 probe, cg05867423, was positively correlated between blood and brain according to data from
347 Hannon et al. tool (r=0.36, p<0.002), with positive correlations also identified in the BECon
348 and IMAGE CPG tools (see Supplementary Tables 6c, 7c, 8c). In addition, cg08455700
349 showed high correlations (r=0.71) between blood and Brodmann area 20 according to the
350 BECon tool (Supplementary Table 7c). None of these probes was related to eQTMs in blood.

351 Regarding chromatin states, we found that the genome-wide significant probe
352 (cg11945228) and the DMR found at childhood were associated with active states (active
353 transcription start site (TSS)-proximal promoter state and a transcribed state at the 5′ and 3′
354 end of genes showing both promoter and enhancer signatures) (Supplementary Table 10). In
355 fact, an enrichment analysis for chromatin states revealed an overrepresentation of active
356 states associated with zinc finger protein genes (ZNF/Rpts) within the most significant CpGs
357 ($p < 1\times10^{-4}$) detected in the prospective meta-analysis (Supplementary Table 11a). In
358 contrast, no consistent enrichment for active states vs repressed states was found based on the
359 most significant CpGs detected in the cross-sectional meta-analysis ($p < 1\times10^{-4}$). However,
360 we observed a significant underrepresentation of active transcription start site (TSS)-proximal
361 promoter states (TssAFlnk), and an overrepresentation of actively-transcribed states (Tx,
362 TxWk) together with inactive quiescent states (Quies) (Supplementary Table 11b). Moreover,
363 according to ENCODE data on several cell lines, including different blood cell types, the
364 DMR (chr15:49,170,042-49,170,244, GRCh37/hg19) is enriched by H3K27Ac histone marks
365 and overlaps with DNAse hypersensitive areas, which are usually associated to active
366 regulatory elements (Supplementary Figure 4a). Hence, according to Chromatin interaction
367 data (in situ Hi-C Chromatin Structure from a lymphoblastoid cell line), the genomic elements

368 comprised in the region involving the two genes associated with the DMR seem to strongly
369 interact with each other (Supplementary Figure 4b).

370 GO, KEGG, and MSigDB analyses revealed no significantly enriched common
371 biological processes, cellular components, molecular functions or pathways for the genes
372 mapped to the probes at $p< 1\times10^{-4}$ in the meta-analyses at birth (n=56) and during childhood
373 (n=104) (see Supplementary Tables 12a-f).

374 Results of the GWAS enrichment analyses for EWAS are presented in
375 **Supplementary Table 13**. Of the 56 probes at $p< 1\times10^{-4}$ in the prospective EWAS meta-
376 analysis, six overlapped with genomic loci previously linked to general psychopathology[16],
377 schizophrenia[45], neuroticism[46], ADHD[47] or anxiety[48] based on GWASs. Of the 104 probes at
378 $p< 1\times10^{-4}$ in our cross-sectional EWAS meta-analysis, 13 (12.5%) overlapped with genomic
379 loci previously linked to these psychiatric outcomes. Most notably, this cross-sectional
380 enrichment analysis prioritized cg08514304 (*TAOK2*), which was among the top 10
381 suggestive hits identified in our cross-sectional EWAS meta-analysis and showed a consistent
382 direction of effect in all cohorts. Finally, regarding the DMR, SNPs within the region
383 comprising the associated genes *SHC4* and *EID1* have been related with psychiatric disorders
384 such as major depressive disorder[49,50], bipolar disorder[51], mood and psychotic disorders[52],
385 obsessive compulsive disorder[53], and schizophrenia[54] (Supplementary Table 14)
386 (Supplementary Figure 4b). Interestingly, both *SHC4* and *EID1* genes are highly expressed in
387 the brain according to GTEx data (Supplementary Figure 4).

388 **Discussion**

389 We conducted the largest epigenome-wide meta-analysis of GPF in childhood, using DNAm
390 assessments at two different time points (birth and childhood). The analyses revealed little
391 evidence for probe-specific associations between DNAm in cord blood or peripheral blood

392 and GPF. However, we did identify a significant DMR in childhood, implicating two relevant
393 genes.

394 On the basis of probe-level genome-wide meta-analyses, we found that lower DNA
395 methylation at cg11945228 at school-age was significantly associated with higher levels of
396 GPF. Cg11945228 is located within the *BRD2* gene, a BET (bromodomains and extra
397 terminal domain) family chromatin adaptor that controls the transcription of a wide range of
398 pro-inflammatory genes[55] and is involved in neural tube closure,[56] neurogenesis,[57] and
399 neuroinflammation.[58] DNAm of the *BRD2* promotor has been implicated in juvenile
400 myoclonic epilepsy, a common adolescent-onset genetic generalized epilepsy syndrome.[59]
401 However, we advise caution when interpreting this specific site because, despite having low
402 variation attributable to heterogeneity across the cohorts, its genome-wide significant
403 association with GPF seems to be driven by one single cohort.

404 With regards to genes with probes at suggestive significance at school-age (*WDR20*,
405 *MOV10*, and *TAOK2)*, these have previously been linked to neurodevelopmental and
406 psychiatric risk, such as autism spectrum disorder (ASD) and schizophrenia.[60–67] Pleiotropy
407 was supported by our cross-sectional GWAS enrichment analyses for EWAS, showing that
408 *TAOK2* overlapped with genomic loci previously linked to schizophrenia,[16,45] as well as
409 obsessive compulsive disorder and bipolar disorder.[16] However, despite these previously
410 established links with mental health outcomes, annotated genes of our overall top hits
411 identified in the EWAS meta-analyses were not enriched for common biological processes,
412 cellular components, molecular functions, or pathways.

413 The significant DMR identified at school-age included 6 CpGs mapped to *SHC4* and
414 *EID1* genes, which are highly expressed in the brain. *SHC4* regulates BDNF-induced MAPK
415 activation[68] and *EID1* plays an important role in the central nervous system[69], being involved

416 in cell proliferation in the brain, synaptic plasticity and memory function. Interestingly,
417 genetic variants in these genes have been previously implicated in multiple psychiatric
418 disorders according to several studies (mostly GWAS), including bipolar disorder[51],
419 obsessive-compulsive disorder[53], mood and psychotic disorders[52], schizophrenia[54], or
420 MDD[49,50,70] (Supplementary Table 14) (Supplementary Figure 4b). The fact that the DMR
421 overlaps with active regulatory elements of these genes and shows evidence of blood-brain
422 concordance for some of the CpGs supports the potential functional relevance of this region.
423 Mechanistic studies will be needed in future to elucidate biological processes underlying the
424 observed link between DNAm in this region and increased risk for multiple psychiatric
425 outcomes. Of interest, despite similar sample sizes and measures (i.e., almost exclusively the
426 CBCL), the top signals were very different between the prospective and cross-sectional
427 EWASs, as evidenced for example by the lack of a correlation between nominally significant
428 sites for these analyses. This low overlap might be due to the temporally dynamic nature of
429 the methylome. DNAm patterns vary substantially over time[71] and can show temporally
430 specific associations with outcomes, including psychiatric symptoms.[20] Unlike an existing
431 EWAS meta-analysis on ADHD symptoms, which showed the strongest signal prospectively
432 at birth compared to childhood,[20] we did not detect any significant prospective associations.
433 This is particularly interesting given the use of largely overlapping samples, suggesting that
434 cord blood DNAm may capture risk for specific psychiatric problems (in this case ADHD)
435 rather than a broader liability to psychopathology.

436 Strengths of this study include the large sample size and the use of DNAm at two
437 different time points (birth and childhood), enabling the assessment of both prospective and
438 cross-sectional associations with GPF. Another important strength is the use of standardized
439 protocols and scripts to fit GPF to the data in a multi-cohort setting. The GPF scores we
440 analyzed were previously found to associate with a module of co-methylated DNAm probes

441 across the genome,[23] suggesting that it is possible to detect biological correlates of GPF using
442 this study's measure. Furthermore, the current study showed that GPF consistently negatively
443 correlated with child cognition across the cohorts as expected based on existing evidence,[7]
444 suggesting that it is capturing a similar, valid construct across the cohorts.

445 However, the current findings should also be interpreted in the context of several
446 limitations. First, given the possibility of residual confounding and reverse causality, the
447 direction of the observed associations cannot be inferred. DNAm might be a marker for
448 unmeasured environmental factors that could affect GPF via independent pathways.
449 Furthermore, children with higher levels of mental health problems may evoke a particular
450 environment,[72] which might affect DNAm. Second, our top hits were unrelated to eQTMs.
451 Future research integrating transcriptomic data will be important for assessing the functional
452 relevance of DNAm changes to gene expression in the brain. Third, because DNAm is tissue
453 specific, our observations in peripheral blood may not reflect DNAm levels in other,
454 potentially more relevant, tissues such as the brain. Despite potential sex differences in mental
455 health problems,[73] the current study did not examine sex-specificity for power reasons.
456 Further, participating cohorts used different normalization pipelines, which may have
457 contributed to cohort differences and influenced our results. **Further, participating cohorts**
458 **used different normalization pipelines, which may have contributed to cohort**
459 **differences and influenced our results. In future, it would be optimal for meta-analytic**
460 **studies to utilize a standardized processing pipeline across all samples. Furthermore, we**
461 **found heterogeneity in CFA parameters, particularly for the specific internalizing and**
462 **externalizing factors (especially in the GLAKU cohort). This precluded us from**
463 **investigating whether, aside from the GPF, DNA methylation patterns also associate**
464 **with variance that is unique to these symptom domains – an interesting question for**
465 **future research.** In future, it would be optimal for meta-analytic studies to utilize a

466 standardized processing pipeline across all samples. Finally, the present findings are based on
467 a predominantly European population and the cohorts are sampled from settings which largely
468 have socialized healthcare, access to mental health services, and different cultural stigma
469 surrounding mental health than other population groups. Future genome-wide studies with
470 larger sample sizes are needed to replicate our findings in other ancestries and in more diverse
471 settings to further characterize DNAm sites associated with GPF.

472 In summary, this large EWAS meta-analysis identified one probe (Cg11945228) for
473 which lower DNAm in childhood was associated with higher levels of GPF. Furthermore, one
474 DMR in childhood was associated with GPF. This DMR included 6 CpGs mapped to the
475 *SHC4* gene that has previously been implicated in multiple types of psychiatric disorders in
476 adulthood. In contrast, no prospective associations were identified with DNAm patterns at
477 birth. The current findings call for a more integrative approach to the study of GPF, using
478 multiple omics sources, including the genome, epigenome, and transcriptome, to achieve a
479 more comprehensive understanding of its biological underpinnings.

480

481 **Data availability**

482 Site-level meta-analytical results will be made publicly available (https://doi.org/......) upon
483 acceptance for publication. For access to cohort-level data, requests can be sent directly to
484 individual studies.

485 **Code availability**

486 Analytical codes can be requested from authors.

487 **Acknowledgements**

488 Acknowledgements for each of the participating studies are listed in the Funding and

489 Acknowledgements supplement.

490 **Compliance with ethical standards**

491 **Conflict of interest** The authors confirm they have no financial relationships with

492 commercial interests to disclose. Funding for each of the participating studies is listed in the

493 Funding and Acknowledgements supplement. There was no editorial direction or censorship

494 from the sponsors.

495 **Ethics** All studies acquired approval from local ethics committees and informed consent was

496 obtained for all participants. Full details are listed in the methods supplement.

497

498

References

499 1. Angold, A., Costello, E. J. & Erkanli, A. Comorbidity. *J. Child Psychol. Psychiatry* **40**, 57–87 (1999).

500 2. Kessler, R. C., Chiu, W. T., Demler, O., Merikangas, K. R. & Walters, E. E. Prevalence, severity, and
501 comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch.*
502 *Gen. Psychiatry* **62**, 617–627 (2005).

503 3. Cuffe, S. P. *et al.* ADHD and Psychiatric Comorbidity: Functional Outcomes in a School-Based
504 Sample of Children. *J. Atten. Disord.* **24**, 1345–1354 (2020).

505 4. Roy-Byrne, P. P. *et al.* Lifetime panic-depression comorbidity in the National Comorbidity Survey.
506 Association with symptoms, impairment, course and help-seeking. *Br. J. Psychiatry J. Ment. Sci.*
507 **176**, 229–235 (2000).

508 5. Lahey, B. B. *et al.* Criterion validity of the general factor of psychopathology in a prospective
509 study of girls. *J. Child Psychol. Psychiatry* **56**, 415–422 (2015).

510 6. Rijlaarsdam, J., Cecil, C. A. M., Buil, J. M., van Lier, P. A. C. & Barker, E. D. Exposure to Bullying
511 and General Psychopathology: A Prospective, Longitudinal Study. *Res. Child Adolesc.*
512 *Psychopathol.* **49**, 727–736 (2021).

513 7. Neumann, A. *et al.* Single Nucleotide Polymorphism Heritability of a General Psychopathology
514 Factor in Children. *J. Am. Acad. Child Adolesc. Psychiatry* **55**, 1038-1045.e4 (2016).

515 8. Caspi, A. & Moffitt, T. E. All for One and One for All: Mental Disorders in One Dimension. *Am. J.*
516 *Psychiatry* **175**, 831–844 (2018).

517 9. Pettersson, E., Lahey, B. B., Larsson, H. & Lichtenstein, P. Criterion Validity and Utility of the
518 General Factor of Psychopathology in Childhood: Predictive Associations With Independently
519 Measured Severe Adverse Mental Health Outcomes in Adolescence. *J. Am. Acad. Child Adolesc.*
520 *Psychiatry* **57**, 372–383 (2018).

521 10. Sallis, H. *et al.* General psychopathology, internalising and externalising in children and functional
522 outcomes in late adolescence. *J. Child Psychol. Psychiatry* **60**, 1183–1190 (2019).

523 11. Brikell, I. *et al.* The contribution of common genetic risk variants for ADHD to a general factor of
524 childhood psychopathology. *Mol. Psychiatry* **25**, 1809–1821 (2020).
525 12. Riglin, L. *et al.* Using Genetics to Examine a General Liability to Childhood Psychopathology.
526 *Behav. Genet.* **50**, 213–220 (2020).
527 13. Brodbeck, J. *et al.* Differential associations between patterns of child maltreatment and
528 comorbidity in adult depressed patients. *J. Affect. Disord.* **230**, 34–41 (2018).
529 14. Caspi, A. *et al.* The p Factor: One General Psychopathology Factor in the Structure of Psychiatric
530 Disorders? *Clin. Psychol. Sci. J. Assoc. Psychol. Sci.* **2**, 119–137 (2014).
531 15. Campbell, M. *et al.* Overlap in genetic risk for cross-disorder vulnerability to mental disorders
532 and genetic risk for altered subcortical brain volumes. *J. Affect. Disord.* **282**, 740–756 (2021).
533 16. Cross-Disorder Group of the Psychiatric Genomics Consortium. Electronic address:
534 plee0@mgh.harvard.edu & Cross-Disorder Group of the Psychiatric Genomics Consortium.
535 Genomic Relationships, Novel Loci, and Pleiotropic Mechanisms across Eight Psychiatric
536 Disorders. *Cell* **179**, 1469-1482.e11 (2019).
537 17. Teschendorff, A. E. & Relton, C. L. Statistical and integrative system-level analysis of DNA
538 methylation data. *Nat. Rev. Genet.* **19**, 129–147 (2018).
539 18. Meaney, M. J. Epigenetics and the biological definition of gene x environment interactions. *Child*
540 *Dev.* **81**, 41–79 (2010).
541 19. Cecil, C. A. M. *et al.* Neonatal DNA methylation and early-onset conduct problems: A genome-
542 wide, prospective study. *Dev. Psychopathol.* **30**, 383–397 (2018).
543 20. Neumann, A. *et al.* Association between DNA methylation and ADHD symptoms from birth to
544 school age: a prospective meta-analysis. *Transl. Psychiatry* **10**, 398 (2020).
545 21. Hannon, E. *et al.* An integrated genetic-epigenetic analysis of schizophrenia: evidence for co-
546 localization of genetic associations and differential DNA methylation. *Genome Biol.* **17**, 176
547 (2016).

548 22. Zhu, Y. *et al.* Genome-wide profiling of DNA methylome and transcriptome in peripheral blood
549 monocytes for major depression: A Monozygotic Discordant Twin Study. *Transl. Psychiatry* **9**, 215
550 (2019).
551 23. Rijlaarsdam, J. *et al.* Genome-wide DNA methylation patterns associated with general
552 psychopathology in children. *J. Psychiatr. Res.* **140**, 214–220 (2021).
553 24. Zhou, W., Laird, P. W. & Shen, H. Comprehensive characterization, annotation and innovative use
554 of Infinium DNA methylation BeadChip probes. *Nucleic Acids Res.* **45**, e22 (2017).
555 25. Solomon, O. *et al.* Comparison of DNA methylation measured by Illumina 450K and EPIC
556 BeadChips in blood of newborns and 14-year-old children. *Epigenetics* **13**, 655–664 (2018).
557 26. Patalay, P. *et al.* A general psychopathology factor in early adolescence. *Br. J. Psychiatry J. Ment.*
558 *Sci.* **207**, 15–22 (2015).
559 27. Gervin, K. *et al.* Systematic evaluation and validation of reference and library selection methods
560 for deconvolution of cord blood DNA methylation data. *Clin. Epigenetics* **11**, 125 (2019).
561 28. Houseman, E. A. *et al.* DNA methylation arrays as surrogate measures of cell mixture
562 distribution. *BMC Bioinformatics* **13**, 86 (2012).
563 29. Salas, L. A. *et al.* An optimized library for reference-based deconvolution of whole-blood
564 biospecimens assayed using the Illumina HumanMethylationEPIC BeadArray. *Genome Biol.* **19**,
565 64 (2018).
566 30. Markunas, C. A. *et al.* Maternal Age at Delivery Is Associated with an Epigenetic Signature in Both
567 Newborns and Adults. *PloS One* **11**, e0156361 (2016).
568 31. Carslake, D., Tynelius, P., van den Berg, G., Davey Smith, G. & Rasmussen, F. Associations of
569 parental age with health and social factors in adult offspring. Methodological pitfalls and
570 possibilities. *Sci. Rep.* **7**, 45278 (2017).
571 32. Rosseel, Y. lavaan: An R Package for Structural Equation Modeling. *J. Stat. Softw.* **48**, 1–36 (2012).
572 33. Willer, C. J., Li, Y. & Abecasis, G. R. METAL: fast and efficient meta-analysis of genomewide
573 association scans. *Bioinforma. Oxf. Engl.* **26**, 2190–2191 (2010).

574 34. Min, J. L., Hemani, G., Davey Smith, G., Relton, C. & Suderman, M. Meffil: efficient normalization
575 and analysis of very large DNA methylation datasets. *Bioinforma. Oxf. Engl.* **34**, 3983–3989
576 (2018).
577 35. Suderman, M. *et al. dmrff: identifying differentially methylated regions efficiently with power*
578 *and control*. 508556 https://www.biorxiv.org/content/10.1101/508556v1 (2018)
579 doi:10.1101/508556.
580 36. Battram, T. *et al.* The EWAS Catalog: a database of epigenome-wide association studies.
581 *Wellcome Open Res.* **7**, 41 (2022).
582 37. Li, M. *et al.* EWAS Atlas: a curated knowledgebase of epigenome-wide association studies.
583 *Nucleic Acids Res.* **47**, D983–D988 (2019).
584 38. Hannon, E. *et al.* Characterizing genetic and environmental influences on variable DNA
585 methylation using monozygotic and dizygotic twins. *PLoS Genet.* **14**, e1007544 (2018).
586 39. Hannon, E., Lunnon, K., Schalkwyk, L. & Mill, J. Interindividual methylomic variation across blood,
587 cortex, and cerebellum: implications for epigenetic studies of neurological and neuropsychiatric
588 phenotypes. *Epigenetics* **10**, 1024–1032 (2015).
589 40. Edgar, R. D., Jones, M. J., Meaney, M. J., Turecki, G. & Kobor, M. S. BECon: a tool for interpreting
590 DNA methylation findings from blood in the context of brain. *Transl. Psychiatry* **7**, e1187 (2017).
591 41. Braun, P. *et al.* 28 - IMAGE-CpG: DEVELOPMENT OF A WEB-BASED SEARCH TOOL FOR GENOME-
592 WIDE DNA METHYLATION CORRELATION BETWEEN LIVE HUMAN BRAIN AND PERIPHERAL
593 TISSUES WITHIN INDIVIDUALS. *Eur. Neuropsychopharmacol.* **29**, S796 (2019).
594 42. Phipson, B., Maksimovic, J. & Oshlack, A. missMethyl: an R package for analyzing data from
595 Illumina's HumanMethylation450 platform. *Bioinforma. Oxf. Engl.* **32**, 286–288 (2016).
596 43. isglobal-brge/EASIER: EwAS: quality control, meta-analysIs and EnRichment version 0.1.2.8 from
597 GitHub. https://rdrr.io/github/isglobal-brge/EASIER/.
598 44. Lawrence, M. *et al.* Software for computing and annotating genomic ranges. *PLoS Comput. Biol.*
599 **9**, e1003118 (2013).

600 45. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from
601 108 schizophrenia-associated genetic loci. *Nature* **511**, 421–427 (2014).
602 46. Nagel, M. *et al.* Meta-analysis of genome-wide association studies for neuroticism in 449,484
603 individuals identifies novel genetic loci and pathways. *Nat. Genet.* **50**, 920–927 (2018).
604 47. Demontis, D. *et al.* Discovery of the first genome-wide significant risk loci for attention
605 deficit/hyperactivity disorder. *Nat. Genet.* **51**, 63–75 (2019).
606 48. Levey, D. F. *et al.* Reproducible Genetic Risk Loci for Anxiety: Results From ∼200,000 Participants
607 in the Million Veteran Program. *Am. J. Psychiatry* **177**, 223–232 (2020).
608 49. Aragam, N., Wang, K.-S. & Pan, Y. Genome-wide association analysis of gender differences in
609 major depressive disorder in the Netherlands NESDA and NTR population-based samples. *J.*
610 *Affect. Disord.* **133**, 516–521 (2011).
611 50. Howard, D. M. *et al.* Genome-wide haplotype-based association analysis of major depressive
612 disorder in Generation Scotland and UK Biobank. *Transl. Psychiatry* **7**, 1263 (2017).
613 51. Budde, M. *et al.* Efficient region-based test strategy uncovers genetic risk factors for functional
614 outcome in bipolar disorder. *Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol.*
615 **29**, 156–170 (2019).
616 52. Blokland, G. A. M. *et al.* Sex-Dependent Shared and Nonshared Genetic Architecture Across
617 Mood and Psychotic Disorders. *Biol. Psychiatry* **91**, 102–117 (2022).
618 53. Boraska, V. *et al.* Genome-wide association analysis of eating disorder-related symptoms,
619 behaviors, and personality traits. *Am. J. Med. Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int.*
620 *Soc. Psychiatr. Genet.* **159B**, 803–811 (2012).
621 54. Goes, F. S. *et al.* Genome-wide association study of schizophrenia in Ashkenazi Jews. *Am. J. Med.*
622 *Genet. Part B Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet.* **168**, 649–659 (2015).
623 55. Wang, N., Wu, R., Tang, D. & Kang, R. The BET family in immunity and disease. *Signal Transduct.*
624 *Target. Ther.* **6**, 23 (2021).

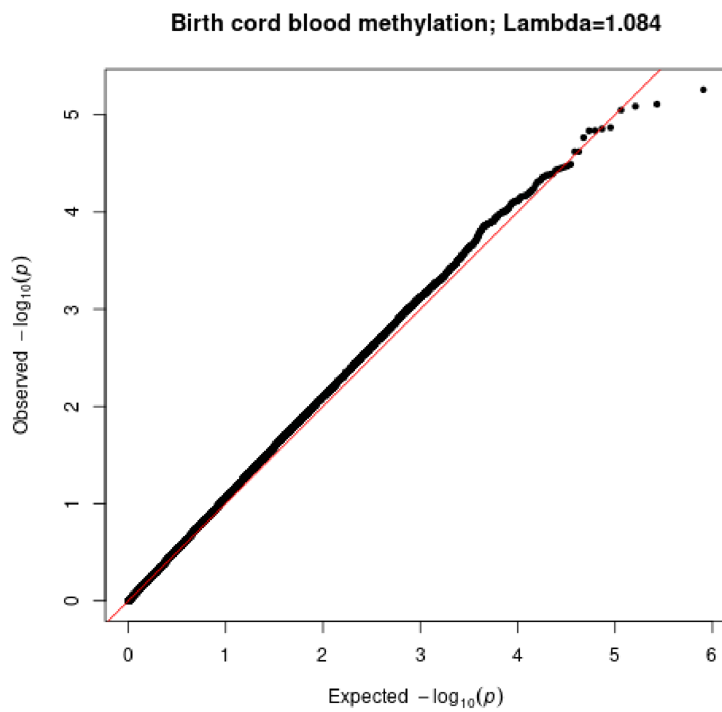
625 56. Gyuris, A. *et al.* The chromatin-targeting protein Brd2 is required for neural tube closure and
626 embryogenesis. *Biochim. Biophys. Acta* **1789**, 413–421 (2009).
627 57. Garcia-Gutierrez, P., Juarez-Vicente, F., Wolgemuth, D. J. & Garcia-Dominguez, M. Pleiotrophin
628 antagonizes Brd2 during neuronal differentiation. *J. Cell Sci.* **127**, 2554–2564 (2014).
629 58. DeMars, K. M., Yang, C. & Candelario-Jalil, E. Neuroprotective effects of targeting BET proteins
630 for degradation with dBET1 in aged mice subjected to ischemic stroke. *Neurochem. Int.* **127**, 94–
631 102 (2019).
632 59. Pathak, S., Miller, J., Morris, E. C., Stewart, W. C. L. & Greenberg, D. A. DNA methylation of the
633 BRD2 promoter is associated with juvenile myoclonic epilepsy in Caucasians. *Epilepsia* **59**, 1011–
634 1019 (2018).
635 60. Wockner, L. F. *et al.* Genome-wide DNA methylation analysis of human brain tissue from
636 schizophrenia patients. *Transl. Psychiatry* **4**, e339 (2014).
637 61. McKinney, B., Ding, Y., Lewis, D. A. & Sweet, R. A. DNA methylation as a putative mechanism for
638 reduced dendritic spine density in the superior temporal gyrus of subjects with schizophrenia.
639 *Transl. Psychiatry* **7**, e1032 (2017).
640 62. Skariah, G. *et al.* Mov10 suppresses retroelements and regulates neuronal development and
641 function in the developing brain. *BMC Biol.* **15**, 54 (2017).
642 63. Vissers, L. E. L. M., Gilissen, C. & Veltman, J. A. Genetic studies in intellectual disability and
643 related disorders. *Nat. Rev. Genet.* **17**, 9–18 (2016).
644 64. Hanson, E. *et al.* The cognitive and behavioral phenotype of the 16p11.2 deletion in a clinically
645 ascertained population. *Biol. Psychiatry* **77**, 785–793 (2015).
646 65. Zufferey, F. *et al.* A 600 kb deletion syndrome at 16p11.2 leads to energy imbalance and
647 neuropsychiatric disorders. *J. Med. Genet.* **49**, 660–668 (2012).
648 66. Weiss, L. A. *et al.* Association between microdeletion and microduplication at 16p11.2 and
649 autism. *N. Engl. J. Med.* **358**, 667–675 (2008).

650 67. Chang, H., Li, L., Li, M. & Xiao, X. Rare and common variants at 16p11.2 are associated with
651 schizophrenia. *Schizophr. Res.* **184**, 105–108 (2017).
652 68. You, Y. *et al.* ShcD interacts with TrkB via its PTB and SH2 domains and regulates BDNF-induced
653 MAPK activation. *BMB Rep.* **43**, 485–490 (2010).
654 69. Liu, R. *et al.* Increased EID1 nuclear translocation impairs synaptic plasticity and memory
655 function associated with pathogenesis of Alzheimer's disease. *Neurobiol. Dis.* **45**, 902–912
656 (2012).
657 70. Sullivan, P. F. *et al.* Genome-wide association for major depressive disorder: a possible role for
658 the presynaptic protein piccolo. *Mol. Psychiatry* **14**, 359–375 (2009).
659 71. Mulder, R. H. *et al.* Epigenome-wide change and variation in DNA methylation in childhood:
660 trajectories from birth to late adolescence. *Hum. Mol. Genet.* **30**, 119–134 (2021).
661 72. Singham, T. *et al.* Concurrent and Longitudinal Contribution of Exposure to Bullying in Childhood
662 to Mental Health: The Role of Vulnerability and Resilience. *JAMA Psychiatry* **74**, 1112–1119
663 (2017).
664 73. Bale, T. L. & Epperson, C. N. Sex as a Biological Variable: Who, What, When, Why, and How.
665 *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* **42**, 386–396 (2017).
666

667

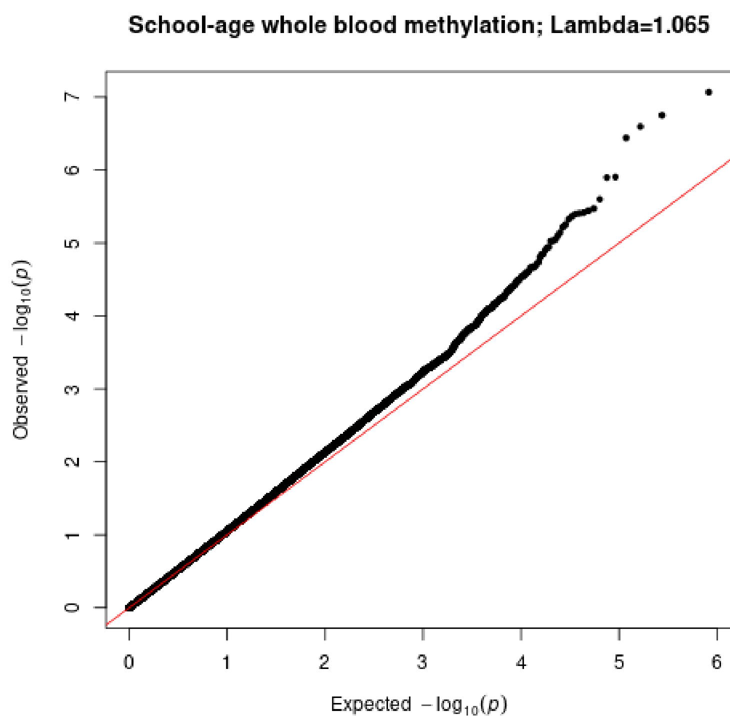
668

669 **Fig. 1. Quantile-quantile plot of the meta-analytic associations of DNA methylation at**
670 **birth and DNA methylation at school-age with general psychopathology.** The diagonal
671 line represents the distribution of the expected *p*-values under the null. Points above the
672 diagonal refer to *p*-values that are lower than expected.



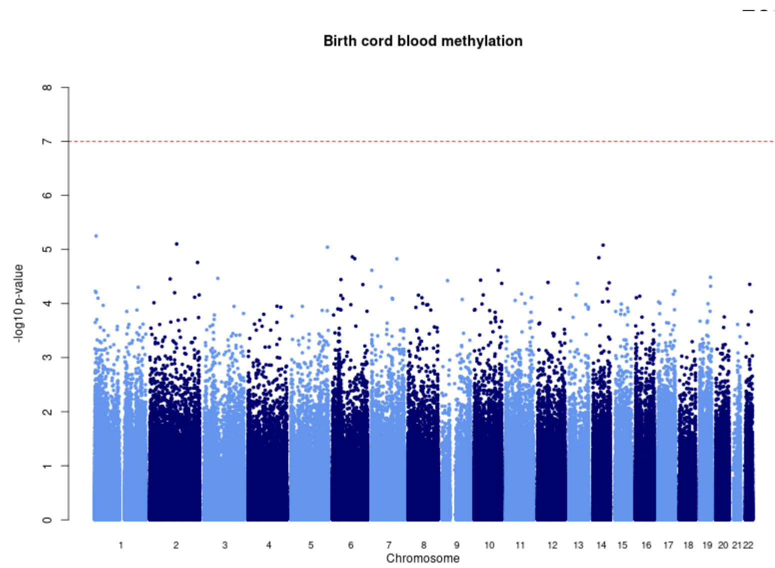
684

685

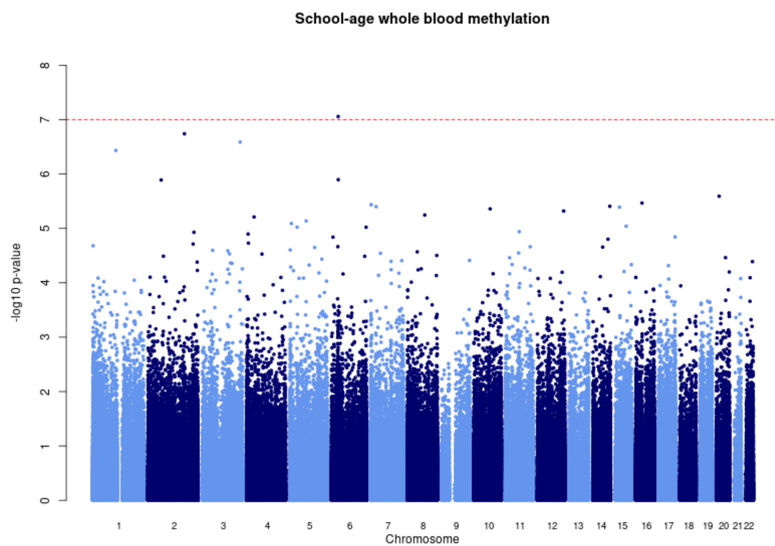


697 **Fig. 2. Manhattan plot of –$\log_{10}$ *p*-values versus CpG position (base pair and**
698 **chromosome) showing meta-analytic associations of DNA methylation at birth and DNA**
699 **methylation at school-age with general psychopathology.** The red line indicates genome-
700 wide significance ($p < 1 \times 10^{-7}$).

701



711



721

Table 1 Cohort characteristics

Cohort	Ancestry/ethnicity	*n*	DNAm age	GPF age	GPF instrument	Effect sizes			λ
						1st Qu	Median	3rd Qu	
Birth EWAS									
ALSPAC	European	643	0	10	DAWBA	-1.10	0.02	1.18	0.98
DCHS	Black African, mixed	151	0	6	CBCL	-4.35	-0.77	2.75	0.95
Generation R	European	1092	0	10	CBCL	-0.77	0.08	1.00	1.01
INMA	European	292	0	9	CBCL	-1.53	0.39	3.03	1.14
Meta		2178				-0.51	0.07	0.77	1.08
Childhood EWAS									
ALSPAC	European	697	7	10	DAWBA	-1.26	0.02	1.28	1.00
Generation R	European	434	10	10	CBCL	-1.51	0.08	1.84	1.03
GLAKU	European	215	12	12	CBCL	-1.66	0.31	2.24	0.91
HELIX	European	779	8	8	CBCL	-1.36	0.32	2.22	0.99

HELIX	Pakistani	65	7	7	CBCL	-4.47	2.52	10.95	1.20
Meta		2190				-0.54	0.18	1.00	1.07

Table 2 DNA methylation at birth and general psychopathology: meta-analytic associations with $p < 1\times10^{-5}$

CpG	Gene	Chr	Position	*n*	B	SE	*p*	Direction	I^2	Heterogeneity *p*-value
cg02084087	*TNFRSF25*	chr1	6526049	2175	4.31	0.95	5.54×10^{-6}	++++	46.2	0.13
cg11777523	*GPR148*	chr2	131485418	2019	9.26	2.07	7.80×10^{-6}	+?++	23.6	0.27
cg14358879	*SLC8A3*	chr14	70655920	2174	-7.82	1.75	8.20×10^{-6}	----	33.7	0.21
cg09437808	-	chr5	176107069	2177	2.46	0.55	8.96×10^{-6}	++++	0	0.47

Chr = chromosome, *n* = number of participants, SE = standard error, Direction = direction of the effect per study (ALSPAC, DCHS, GENR, INMA) in alphabetical order (+ = positive direction, - = negative direction, ? = not present); I^2 = heterogeneity statistic reflecting the variation attributable to heterogeneity across studies (high values suggest high heterogeneity)

Note. Effect estimates (B) represent the SD increase in GPF for each increase of 100% in DNAm.

Table 3 DNA methylation at school-age and general psychopathology: meta-analytic associations with $p < 1 \times 10^{-5}$

CpG	Gene	Chr	Position	*n*	B	SE	*p*	Direction	I^2	Heterogeneity *p*-value
cg11945228	*BRD2*	chr6	32940368	2173	-37.00	6.91	8.58×10^{-8}	----+	0	0.53
cg18862005	-	chr2	177940863	1972	3.78	0.72	1.78x10-^{7}	++?++	0	0.64
cg22691524	-	chr3	185300576	2180	7.84	1.52	2.54×10^{-7}	+++++	0	0.47
cg00719568	*MOV10*	chr1	113239645	2184	4.20	0.83	3.62×10^{-7}	+++++	0	0.67
cg09040034	*KIFC1*	chr6	33362567	1966	4.89	1.01	1.25×10^{-6}	++?+-	40	0.17
cg24514921	*VPS54*	chr2	64246311	2184	12.17	2.51	1.27×10^{-6}	++++-	0	0.45
cg25182716	-	chr20	13622875	2178	6.42	1.36	2.52×10^{-6}	+++++	0	0.76
cg08514304	*TAOK2*	chr16	29994437	2175	6.88	1.48	3.36×10^{-6}	+++++	0	0.59
cg00470277	-	chr7	2669915	2185	4.55	0.98	3.62×10^{-6}	+++++	0	0.99
cg26353764	*WDR20*	chr14	102660055	2187	6.82	1.48	3.86×10^{-6}	+++++	43.7	0.13
cg27009703	*HOXA9*	chr7	27204894	2121	-8.58	1.86	3.93×10^{-6}	-----	0	0.52
cg12492087	*ZFP106*	chr15	42749885	2178	6.39	1.39	4.01×10^{-6}	+++++	0	0.92

cg18436008	-	chr10	80535327	2176	5.48	1.19	4.31x10^{-6}	+++++	0	0.56
cg17281031	-	chr12	128223216	2186	4.50	0.98	4.73x10^{-6}	+++++	14.6	0.32
cg08327106	*RALYL*	chr8	85094842	2175	8.60	1.89	5.60x10^{-6}	+++++	0	0.66
cg11236841	-	chr4	35567978	2175	4.67	1.03	6.08x10^{-6}	+++++	54.3	0.07
cg21525176	*LHFPL2*	chr5	77906752	2182	6.09	1.36	7.19x10^{-6}	++-++	59.2	0.04
cg26420013	*NSUN2;SRD5A1*	chr5	6632020	2189	2.44	0.55	8.03x10^{-6}	+++++	56.1	0.06
cg17087232	*MAN2C1*	chr15	75651821	2187	3.45	0.78	9.00x10^{-6}	+++++	0	0.98
cg16360861	*RAI14*	chr5	34684597	2175	5.12	1.16	9.36x10^{-6}	+++++	0	0.53
cg00737264	*SMOC2*	chr6	169049498	2188	1.88	0.42	9.40x10^{-6}	+-+++	41.3	0.15

Chr = chromosome, *n* = number of participants, SE = standard error, Direction = direction of the effect per study (ALSPAC, GENR, GLAKU, HELIX, HELIX-Pakistani) in alphabetical order (+ = positive direction, - = negative direction, ? = not present); I^2 = heterogeneity statistic reflecting the variation attributable to heterogeneity across studies (high values suggest high heterogeneity)

Note. Effect estimates (B) represent the SD increase in GPF for each increase of 100% in DNAm.

Supplementary material:

SUPPLEMENTARY METHODS

Supplementary material: 36

SUPPLEMENTARY METHODS 36

1. *Analytical models* *38*

2. *Avon Longitudinal Study of Parents and Children (ALSPAC)* *39*

3. *Drakenstein Child Health Study (DCHS)* *43*

4. *Generation R Study (Generation R)* *46*

5. *Glycyrrhizin in Licorice (GLAKU)* *48*

6. *Human Early Life Exposome (HELIX)* *52*

7. *INfancia y Medio Ambiente (INMA)* *56*

1. Analytical models

***Prospective model*: *DNA methylation at birth* and the general psychopathology factor**

Outcome	**Exposure**	**Covariates**
General psychopathology factor	DNA methylation at birth	Covariates*: maternal smoking status, gestational age, sex, child age at outcome assessment, estimated cell proportions (*Gervin et al., 2019*), batch (optional), maternal age at birth, maternal educational level, ancestry (optional), selection factors (optional)

***Cross-sectional model*: *Childhood DNA methylation* and the general psychopathology factor**

Outcome	**Exposure**	**Covariates**
General psychopathology factor	DNA methylation at childhood	Covariates*: maternal smoking status, gestational age, sex, child age at outcome assessment, estimated cell proportions (*Houseman et al*., 2012), batch (optional), maternal age at birth, maternal educational level, ancestry (optional), selection factors (optional)

***Maternal smoking status, gestational age, sex, ancestry, maternal age, maternal educational level, estimated cell proportions, and child age were considered potential confounders given previous evidence on their association with offspring DNA methylation patterns** *(Alfano et al., 2019; Chu & Yang, 2017; Cosin-Tomas et al., 2022; Gervin et al., 2016; Houseman et al., 2012; Markunas et al., 2016; Merid et al., 2020; Mulder et al., 2021; Yousefi et al., 2015)* **and/or cognitive and psychiatric outcomes** *(Ashford et al., 2008; Carslake et al., 2017; Meyrose et al., 2018; Moster et al., 2008; Riecher-Rössler, 2017; Solmi et al., 2022).*

2. Avon Longitudinal Study of Parents and Children (ALSPAC)

Design and study population: Pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992 were invited to take part in the study (Boyd et al., 2013; Fraser et al., 2013). The initial number of pregnancies enrolled was 14,541 (for these at least one questionnaire has been returned or a "Children in Focus" clinic had been attended by 19/07/99). Of these initial pregnancies, there was a total of 14,676 foetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age. When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join the study originally. As a result, when considering variables collected from the age of seven onwards (and potentially abstracted from obstetric notes) there are data available for more than the 14,541 pregnancies mentioned above. The number of new pregnancies not in the initial sample (known as Phase I enrolment) that are currently represented on the built files and reflecting enrolment status at the age of 24 is 913 (456, 262 and 195 recruited during Phases II, III and IV respectively), resulting in an additional 913 children being enrolled. The phases of enrolment are described in more detail in the cohort profile paper and its update (Boyd et al., 2013; Fraser et al., 2013; Northstone et al., 2019). The total sample size for analyses using any data collected after the age of seven is therefore 15,454 pregnancies, resulting in 15,589 foetuses. Of these 14,901 were alive at 1 year of age. A 10% sample of the ALSPAC cohort, known as the Children in Focus (CiF) group, attended clinics at the University of Bristol at various time intervals between 4 to 61 months of age. The CiF group were chosen at random from the last 6 months of ALSPAC births (1432 families attended at least one clinic). Excluded were those mothers who had moved out of the area or were lost to follow-up, and those partaking in another study of infant development in Avon.

Please note that the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool" and reference the following webpage: http://www.bristol.ac.uk/alspac/researchers/our-data/.

Consent and ethical approval: Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

DNA methylation measurements: As part of the Accessible Resources for Integrated Epigenomic Studies (ARIES, http://www.ariesepigenomics.org.uk/) project, DNA methylation was generated for 1018 mother-offspring pairs from the ALSPAC cohort, using the Infinium HumanMethylation450 BeadChip array (Illumina Inc., San Diego, United States). ARIES participants were selected based on availability of DNA samples at two time points for the mother (antenatal and at follow-up when the offspring were adolescents) and at three time points for the offspring (neonatal, childhood (age 7), and adolescence (age 17)). The current study used child cord blood at birth and whole blood at age 7.

Generation of methylation data and pre-processing methods: Methods for methylation measurements in ALSPAC have been described previously (Relton et al., 2015). Briefly, cord blood was collected according to standard procedures. DNA methylation assays and data pre-processing was performed at the University of Bristol as part of the ARIES project. DNA was extracted using standard protocol and was bisulfite-converted using the Zymo EZ DNA MethylationTM kit (Zymo, Irvine, CA). DNA methylation was then measured using the Infinium HM450 BeadChip assay (Illumina Inc, San Diego, CA), according to the standard protocol. Arrays were scanned using an Illumina iScan. An initial review of data quality was assessed using GenomeStudio (version 2011.1). A semi-random approach (sampling criteria were in place to ensure that all time points were represented on each array) was used to distribute ARIES samples across slides to minimize the possibility of potential confounding by batch. Data were normalised using the meffil R package (Min, Hemani, Davey Smith, Relton, & Suderman, 2018) using the functional normalisation approach.

Child psychopathology data: Offspring psychopathology was assessed using the parent version of the Development and Well-being Assessment (DAWBA; R. Goodman, Ford, Richards, Gatward, & Meltzer, 2000) at the age of 10 years. The DAWBA band computer prediction variables indicate the probability of disorder in 6 categories, ranging from very unlikely (<0.1%) to probable (>70%). Variables based on the ICD-10 and DSM-IV were used to assess ADHD, Conduct Disorder, Depression, Generalized Anxiety Disorder, Obsessive Compulsive Disorder Social Phobia and Specific Phobia and Oppositional Defiant Disorder (A. Goodman, Heiervang, Collishaw, & Goodman, 2011).The DAWBA band variable for Separation Anxiety was based on the DSM-IV only

Cell type correction: Estimated cell type proportion types were obtained using the houseman method (Houseman et al., 2012) with a cord blood reference panel (Gervin et al., 2019) for the prospective analysis and a whole-blood reference panel (Reinius et al., 2012) for the cross-sectional analysis.

Batch correction: Batch effects were accounted for by adjusting for 20 surrogate variables, that were generated using the R package SVA (Leek et al., 2019).

Ancestry/ethnicity: European ancestry was detected by multidimensional scaling analysis using child GWAS data (Gaunt et al., 2016).

Smoking during pregnancy: Maternal smoking during pregnancy was assessed as a categorical variable representing 0 = no smoking during pregnancy, 2 = Stopped before the second trimester of pregnancy and 3 = Smoking in the third trimester or throughout pregnancy.

Gestational age: Gestational age was calculated (in days) based on the date of the mother's last menstrual period (LMP) when the mother was certain of this, but for uncertain LMPs and conflicts with clinical assessment the ultrasound assessment was used. Where maternal report and ultrasound assessment conflicted, an experienced obstetrician reviewed clinical records and made a best estimate.

Child sex: Offspring biological sex at birth was taken from obstetric records.

Child age at behavioral assessment: Child age at the completion of the DAWBA was calculated based on the children's date of birth and the date of DAWBA completion.

Maternal age: Continuous (years).

Maternal education: Maternal education was assessed in the third trimester of pregnancy and coded as a categorical variable representing: high education = advanced-level school-leaving certificate (post-16)/degree, medium education = ordinary-level school-leaving certificate (at 16) and low education = vocational/certificate of secondary education (at 16, equivalent to lower grades of ordinary-level)/none.

Child cognition (external variable): Child IQ was assessed using the WISC-III UK at the age of 8.5 years (Wechsler, Golombok, & Rust, 1992).

Deviations from analysis plan: Sample plate correction in ALSPAC led to model convergence errors because of small batches in ALSPAC. Therefore surrogate variable analysis was used to correct for technical variation instead. Further, estimated cell proportions in ARIES cord blood were normalised using the R-package meffil (Suderman, Hemani, & Min, 2019) instead of the FlowSorted.CordBloodCombined.450k R-package (Salas, Gervin, & Jones, 2020).

3. *Drakenstein Child Health Study (DCHS)*

Design and study population: Drakenstein Child Health Study (DCHS). The DCHS, a population-based birth cohort,has been described previously (Zar, Barnett, Myer, Stein, & Nicol, 2015). Mothers were enrolled prenatally in their second trimester and followed through pregnancy at two primary care clinics serving two distinct populations (predominantly black African ancestry or predominantly mixed ancestry). Mother-child pairs were followed from birth and infants enrolled in the DCHS were followed until at least five years of age (Zar et al., 2015). All births occurred at a single, central facility, Paarl Hospital. The present study is based on children from the DCHS with DNA methylation data from cord blood, genotyping data, and information on psychopathology factors and covariates.

Consent and ethical approval: Ethical approval for human subjects' research was obtained from the Human Research Ethics Committee of the Faculty of Health Sciences of University of Cape Town (HREC UCT REF 401/2009; HREC UCT REF 525/2012). Written informed consent was signed by the mothers on behalf of herself and her infant for participation in this study.

DNA methylation measurements: DNA was isolated from cord blood samples that were collected at time of delivery (Morin et al., 2017). DNA methylation was assessed with the Illumina Infinium HumanMethylation450 BeadChips (n=156) and the MethylationEPIC BeadChips (n=160).

Pre-processing and statistics were done using R 3.5.1 (https://www.r-project.org/). Raw iDat files were imported to RStudio where intensity values were converted into beta values. The 450K and EPIC datasets were then combined using the minfi package (Aryee et al., 2014) resulting in 316 samples and 453,093 probes. Background subtraction, color correction and normalization were performed using the preprocessFunnorm function (Fortin et al., 2014).

Samples were determined to be outliers if detected using two or more of the following methods: detectOutlier function from the lumi package (Du, Kibbe, & Lin, 2008), Hannum et al. (2013) method using the locFDR package (https://cran.r-project.org/package=locfdr) and both the outlyx and pfilter functions from the watermelon package (Pidsley et al., 2013). However, no samples we detected in more than one method and so none were removed for this reason. Samples containing maternal blood contamination (n = 33) were removed (Morin et al., 2017). After the completion of pre-processing technical replicates (n = 7) and samples where reported sex did not match sex chromosome methylation signatures (n = 3) were removed leaving a total of 273 samples remaining for downstream analysis.

This dataset contains 59 probes which detect single nucleotide polymorphisms for quality control purposes and so once observed, were removed. Probes with NAs in ≥ 1% of samples or had a detection p value ≥ 1x10-16 in ≥ 1% of samples were removed (n = 10,868). Probes which bind to the sex chromosomes were removed due to the distribution differences observed (n = 9,896). Probes whose sequence contains a SNP either at the CpG site being measured or at the site of the single base pair extension with a minor allele frequency ≥ 1% (Pidsley et al., 2013; Price et al., 2013) were removed (n = 13,598). Autosomal probes which were in silico predicted to non-specifically bind to sex chromosomes in the genome were also removed (n = 9,698) leaving a total of 409,033 probes remaining for downstream analysis (Pidsley et al., 2013; Price et al., 2013).

Child psychopathology data: Psychopathology symptoms were assessed with the Child Behavior Checklist 6-18 (CBCL/6-18), a validated and widely used parental assessment of a child's behavioral and emotional problems (Achenbach & Rescorla, 2001). Mothers completed questions about a range of emotional and behavioral problems of the child in the past six months on a three-point scale (0=not true, 1=somewhat true, 2=very true).

Cell type correction: Seven default cell types as mentioned in analysis plan. Cord blood cell type composition was predicted using the most recent cord blood reference data set (Gervin et al., 2019) and the IDOL algorithm and probe selection (Koestler et al. 2016).

Batch correction: Batch effects were removed using ComBat from the R package sva (Leek et al., 2019).

Ancestry/ethnicity: 5 genetic principal components included in the model to adjust for population stratification

Smoking during pregnancy: Smoking during pregnancy was assessed based on maternal urine cotinine levels at time of enrollment (second trimester). Passive smokers (exposed in environment but did not smoke themselves, cotinine concentrations >=10 -499 ng/ml) and no smoke exposure (<10 ng/ml) were classified as non-smokers. Active smokers (cotinine concentrations >= 500ng/ml) were classified as smokers. "[0] Non-smokers", "[1] Smokers".

Gestational age: Gestational age was recorded from ultrasound measurements in the second trimester of pregnancy. In cases where no ultrasound measurement was available, the expected date of delivery was calculated using symphysis-fundal height, recorded by trained clinical staff at enrolment, or date of last normal menstrual period.

Child sex: "[1] female", "[2] male"

Child age at behavioral assessment: Continuous (years).

Maternal age: Continuous (years).

Maternal education: "[0] primary", "[1] some secondary", "[2] completed secondary", "[3] any tertiary"

Child cognition (external variable): Child cognitive development was assessed using the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-IV), where cognition was represented by a full-scale IQ (FSIQ) composite score derived from performance across 6 subtests covering verbal and non-verbal areas of cognition; including verbal comprehension, fluid reasoning, visual-spatial ability, processing speed and working memory.

4. Generation R Study (Generation R)

Design and study population: The Generation R Study is a population-based prospective cohort study (Kooijman et al., 2016). All pregnant women living in Rotterdam, the Netherlands, with an expected delivery date between April 2002 and January 2006 were invited to participate. These women and their children have been followed at regular intervals since recruitment. For the current study, only children of European ancestry were included.

Consent and ethical approval: All parents gave informed consent for their children's participation. The Generation R Study is conducted in accordance with the World Medical Association Declaration of Helsinki and study protocols have been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam.

DNA methylation measurements: Preparation and normalization of the Illumina Infinium® HumanMethylation450 BeadChip array data was performed according to the CPACOR workflow using the software package R. In detail, the idat files were read using the *minfi* package. Probes that had a detection p-value above background (based on sum of methylated and unmethylated intensity values) > or equal to 1E-16 were set to missing per array. Next, the intensity values were stratified by autosomal and non-autosomal probes and quantile normalized for each of the six probe type categories separately: type II red/green, type I methylated red/green and type I unmethylated red/green. Beta values were calculated as proportion of methylated intensity value on the sum of methylated+unmethylated+100 intensities. Arrays with observed technical problems such as failed bisulfite conversion, hybridization or extension, as well as arrays with a mismatch between sex of the proband and sex determined by the

chromosome X and Y probe intensities were removed from subsequent analyses. Additionally, only arrays with a call rate > 95% per sample were processed further. The final dataset contained information on 458,563 CpGs for 1,396 samples at birth and 464 samples at age 10.

Child psychopathology data: Psychopathology symptoms were assessed with the Child Behavior Checklist 6-18 (CBCL/6-18), a validated and widely used parental assessment of a child's behavioral and emotional problems (Achenbach & Rescorla, 2001). Mothers completed questions about a range of emotional and behavioral problems of the child in the past six months on a three-point scale (0=not true, 1=somewhat true, 2=very true).

Cell type correction: Cell counts estimated using the Gervin et al. (2019) (DNAm data at birth) or Houseman et al. (2012) (DNAm data at age 10) blood reference panels were included as covariates.

Batch correction: Adjustment for batch effects was done by including sample plate as a covariate.

Ancestry/ethnicity: Ancestry came from child GWAS data. All children with DNA methylation data were of European ancestry.

Smoking during pregnancy: Smoking during pregnancy was assessed with postal questionnaires in early pregnancy (Gestational Age <18 weeks), mid pregnancy (Gestational Age 18-25 weeks) and late pregnancy (Gestational Age>25 weeks). It was classified in three categories: “Never smoked during pregnancy”, “Quit when pregnancy was known” and “Continued during pregnancy”.

Gestational age: (continuous, weeks). Gestational age at birth was established by fetal ultrasound examination

Child sex: (0 = female, 1 = male). Child sex was obtained from midwife and hospital registries at birth.

Child age at behavioral assessment: Continuous (years).

Maternal age: Continuous (years, at intake).

Maternal education: Maternal educational level was based on self-reported levels of education during pregnancy. It was classified following the definition of Statistics Netherlands into three-level ordinal categories: 0= high (higher vocational training or higher academic education), 1= medium (>three years general secondary school); 2= low (lower vocational training or three or less years general secondary school).

Child cognition (external variable): Child cognition was assessed using a nonverbal IQ test when children were 5–7 years old. Two subtests of the Snijders-Oomen Niet-verbale intelligentie test, 2.5-7- revisie (SON-R 2.5-7; Tellegen, Winkel, Wijnberg-Williams, & Laros, 2005) were administered, including "Mosaics" (spatial insight) and "Categories" (abstract reasoning abilities).

5. Glycyrrhizin in Licorice (GLAKU)

Design and study population:

The adolescents of the Glaku (Glycyrrhizin in Licorice) cohort came from an urban community-based cohort comprising 1049 infants of European descent born between March and November 1998 in Helsinki, Finland (Strandberg, Jarvenpaa, Vanhanen, & McKeigue, 2001). In 2009–2011, initial cohort members who had given permission to be contacted and whose addresses were traceable (*N* = 920, 87.7% of the original cohort in 1998) were invited to a follow-up, of which 692 (75.2%) could be contacted by phone (mothers of the adolescents). Of them, 451 (65.2% of those who could be contacted by phone, 49% of the invited) participated in a follow-up at a mean age of 12.3 years (SD = 0.5, range 11.0–13.2 years).

Consent and ethical approval: Informed consent was obtained from all participants. The study protocol was approved by the ethical committees of the City of Helsinki and the Uusimaa Hospital District.

DNA methylation measurements:

DNA was extracted from child blood samples collected through venepuncture at the mean age of 12.3 years (range 11.1-13.2 years).

DNA was extracted at the National Institute for Health and Welfare, Helsinki, Finland and the Department of Medical and Clinical Genetics, University of Helsinki, Finland [LJMT1] and methylation analyses were performed at the Max Planck Institute in Munich, Germany. DNA was bisulphite-converted using the EZ-96 DNA Methylation kit (Zymo Research). Genome-wide methylation status of over 850 000 CpG sites was measured using the Infinium Methylation EPIC array (Illumina Inc., San Diego, USA) according to the standard protocol in 240 blood samples. The arrays were scanned using the iScan System (Illumina Inc., San Diego, USA). The quality control pipeline was set up using the R-package minfi. Methylation beta-values were normalized using the funnorm function.One IDs showed density artefacts after normalization and was removed from further analysis. We excluded any probes on chromosome X or Y, probes containing SNPs and cross-hybridizing probes according to Chen (Chen et al., 2013), Price (Price et al., 2013) and McCartney (McCartney et al., 2016). Furthermore, any CpGs with a detection p-value > 0.01 in at least 25% of the samples were excluded. The final dataset contains 812,943 CpGs and 239 IDs. We used ComBat to check and adjust for the batch effects.

Genotyping was performed on Illumina Human OmniExpress Exome 1.2 bead chip (Illumina Inc., San Diego, CA) at the Tartu University, Estonia in September 2014 according to the standard protocols. Genomic coverage was extended by imputation using the 1000 Genomes Phase I integrated variant set (v3/April 2012; NCBI build 37/hg19) as the reference sample and IMPUTE2 software. Before imputing the following QC, filters were applied: SNP clustering probability for each genotype > 95%, Call rate > 95% individuals and markers (99% for markers with MAF

< 5%), MAF > 1%, HWE p > 1*10–6. Moreover, heterozygosity, sex check, and relatedness checks were performed and any discrepancies were removed (N = 2).

Child psychopathology data: Psychopathology symptoms were assessed with the Child Behavior Checklist 6-18 (CBCL/6-18), a validated and widely used parental assessment of a child's behavioral and emotional problems (Achenbach & Rescorla, 2001). Mothers completed questions about a range of emotional and behavioral problems of the child in the past six months on a three-point scale (0=not true, 1=somewhat true, 2=very true).

Cell type correction: The Houseman method (Houseman et al., 2012) was applied with Reinius reference data (Reinius et al., 2012) using the estimateCellCounts function from the Minfi package (Jaffe & Irizarry, 2014) in R (https://www.r-project.org/) to estimate the proportions of six white blood cell subtypes (CD4+ T-lymphocytes, CD8+ T-lymphocytes, NK (natural killer) cells, B-lymphocytes, monocytes and granulocytes).

Batch correction: We used ComBat to check and adjust for the batch effects.

Ancestry/ethnicity: We performed multi-dimensional scaling (MDS) analysis on the identity by state matrix of quality-controlled genotypes. The first three components depicted the origin admixture and were included as covariates in the regression analyses.

Smoking during pregnancy: was self-reported and categorized as yes or no.

Gestational age: was based on ultrasound scans and derived from the birth register.

Child sex: was derived from the Finnish social security number.

Child age at behavioral assessment: Continuous (years), calculated from the dates of CBCL assessment and birth date.

Maternal age: Continuous (years), derived from the birth register.

Maternal education: was assessed by question "What is the highest education level you have achieved" with 8 categories: 1=four-to-eight-year primary school in the former Finnish school system, 2=nine-year primary+lower secondary school in the current Finnish school system, 3=upper secondary education, 4=post-secondary degree, 5=bachelor's degree, 6=master's degree, 7=doctoral dissertation or 8=some other education. There were no occurrences of the categories 1 nor 8 in the data. Since gaining the primary and lower secondary education takes ~9 years in the current Finnish school system, the maternal education level 2 in Glaku would probably correspond to the ISCED levels 0-2 ('low' maternal education in the study plan). However, in the data there were only 4 mothers with the maternal education level 2. Therefore, we combined the maternal education levels 2 and 3. Furthermore, the cumulative years of schooling for upper secondary education, post-degree, bachelor's degree and master's degree are about 12, 14, 15-16, 17-18 and 22, respectively. For the analysis, the categories 2-3 were assigned to 0, categories 4-5 to 1 and categories 6-7 to 2. The resulting frequencies of these categories were 36, 101 and 78, respectively.

Child cognition (external variable):

Children were administered two subtests tapping on verbal abilities (Similarities, Vocabulary) and two subtests tapping on non-verbal abilities (Block Design and Picture Arrangement) from the Finnish translation of the Wechsler Intelligence Scales for Children, 3rd Edition (WISC-III). The age and sex standardized scores were summed and converted to z-scores based on the observed distribution to produce estimated IQ.

6. Human Early Life Exposome (HELIX)

Design and study population: The present study used data from the Human Early Life Exposome Study (HELIX; https://www.projecthelix.eu/), a collaborative project across six established and ongoing longitudinal population-based birth cohort studies in six European countries (Maitre et al., 2018). The project counts with a harmonization protocol for exposures and phenotypes. For this particular analysis, data from six different HELIX cohorts were used: BIB (United Kingdom), EDEN (France), KANC (Lithuania), INMA (Spain), MOBA (Norway) (Magnus et al., 2016), and RHEA (Greece). From the dataset we only included children of European ancestry (selected by genetic background data). The number of participants with both blood DNA methylation (Infinium®HumanMethylation450 BeadChip) and phenotype data (CBCL test) at the age of 7-9 years was 779. Models were adjusted for 20 GWAS PCs to account for genetic substratitification within Europe.

In parallel, the same analysis was conducted with participants of Pakistani ancestry only (selected by genetic background data). In this case, the number of participants with both DNA methylation and phenotype data is 74. For this particular analysis we did not adjust for PCs since all participants are from the same cohort (BIB, United Kingdom).

Consent and ethical approval: Prior to the start of HELIX, all six cohorts on which HELIX is based had been in existence for some years, had undergone the required evaluation by national ethics committees and had obtained all the required permissions for their cohort recruitment and follow-up visits. Each cohort also confirmed that relevant informed consent and approval were in place for secondary use of data from pre-existing data. The work in HELIX was covered by new ethics approvals in each country, and at enrolment in the HELIX subcohort and panel studies participants were asked to sign an informed consent form for the specific HELIX work including clinical examination and biospecimen collection and analysis. An Ethics Task Force was established to support the HELIX project on ethical issues, for advice on the project's ethical compliance, identification and alerting to changes in legislation where applicable.

Specific procedures are in place within HELIX to safeguard the privacy of study subjects and confidentiality of data. First, any reported study results pertain to analyses of aggregate data; no variables or combination of variables that can identify an individual will be associated with any published or unpublished report of this study. Primary databases with personal information (such as geocodes, dates, questionnaires or health outcomes) have been stored on separate computers with personal identifiers removed. Subjects are identified by a unique study number, linking all basic data required for the study. The master key file linking the study numbers with personal identifiers is maintained in each cohort. For the dataset analysis, all information that enables identification of an individual (dates, geocodes, etc) is removed before distribution of datasets to the researchers. All data exchanges will adhere to the most up-to-date EU and national data protection regulations.

DNA methylation measurements: The following procedure was conducted in the same lab for all the samples from the different cohorts comprising HELIX, which were previously randomized. DNA was obtained from buffy coat collected in EDTA tubes at age 7-9y. Briefly, DNA was extracted using the Chemagen kit (Perkin Elmer) in batches of 12 samples. Samples were extracted by cohort and following their position in the original boxes. DNA concentration was determined in a NanoDrop 1000 UV-Vis Spectrophotometer (ThermoScientific) and with Quant-iT™ PicoGreen™ dsDNA Assay Kit (Life Technologies). DNA methyaltion was assessed with the Infinium HumanMethylation450 beadchip from Illumina, following manufacturer's protocol. Briefly, 700 ng of DNA were bisulfite-converted using the EZ 96-DNA methylation kit following the manufacturer's standard protocol, and DNA methylation measured using the Infinium protocol. A HapMap sample was included in each plate. In addition 24 HELIX inter-plate duplicates were included. Samples were randomized taking into account cohort, sex and panel. Samples from the panel study (samples of the same subject obtained at two time points) were processed in the same array. Two samples were repeated due to their overall low quality. The final number of analyzed samples was 1,361. DNA methylation data were pre-processed using the minfi package (Aryee et al. 2014). Following guidelines of Lehne work (Lehne et al., 2015), we increased the stringency of the detection p-value threshold to 10E-16 and probes not reaching a 98% call rate were excluded. Two samples were filtered due to overall quality: one had a call rate <98% and the other did not pass QC parameters of the MethylAid package (van Iterson et al., 2014). Then, data was normalized with the functional

normalization method, which also includes Noob background subtraction and dye-bias correction (Triche, Weisenberger, Van Den Berg, Laird, & Siegmund, 2013). After that, several quality control checks were performed. First, we checked sex consistency using the shinyMethyl package (Fortin et al., 2014) and two samples were excluded. Genetic consistency of duplicates and samples from the same participant was checked with the 450k genotypes. In addition, genetic consistency was evaluated in those samples that had GWAS data and two of them were excluded. Centered-correlation was around 0 for unrelated samples and around 0.8 for duplicates and panel samples. Principal component analysis showed no differential clusters, however a degree of grouping within the cluster was observed for some biological variables (sex, cohort) and for some technical variables. Because of this, we further used COMBAT algorithm (Johnson, Li, & Rabinovic, 2007) to adjust for potential batch effects, using slide as the major known technical bias. Duplicated samples and HapMap samples were removed as well as control probes, probes designed to detect SNPs and probes to measures methylation levels at non-CpG sites. The final dataset for this analysis consisted of 853 HELIX subjects (779 of European ancestry +74 of Pakistani ancestry) with phenotypic data and covariates and 480071 probes.

Child psychopathology data: Psychopathology symptoms were assessed with the Child Behavior Checklist 6-18 (CBCL/6-18), a validated and widely used parental assessment of a child's behavioral and emotional problems (Achenbach & Rescorla, 2001). Mothers completed questions about a range of emotional and behavioral problems of the child in the past six months on a three-point scale (0=not true, 1=somewhat true, 2=very true).

Cell type correction: blood reference panel with 6 cell types by Houseman et al. (2012).

Batch correction: Potential batch effects were adjusted for by the COMBAT algorithm (Johnson et al., 2007), using slide as the major known technical bias.

Ancestry/ethnicity: In the analysis with participants of European ancestry, models were adjusted for the first 20 genetic principal components (PCs) to adjust for population stratification. Since the PCs also capture variation due to cohort, we did not adjust for a cohort variable to avoid multicollinearity.

Smoking during pregnancy: three-level ordinal category (never smoked during pregnancy, smoked during first trimester only, smoked after first trimester/sustained smoking)

Gestational age: Gestational age (continuous, weeks) was established by combination of the variables indicated below and is equal to: - e3_galmp if available, - OR e3_gaultr if e3_galmp not available, - OR e3_gama if e3_galmp and e3_gaultr not available.

e3_galmp: LMP-based GA (date of delivery - date of LMP)/7 (without rounding), where LMP is last menstrual period; e3_galultr: gestational age estimated using ultrasound measurements if there were performed before 20 weeks of gestation: (date of delivery-date of conception estimated by US)/7; e3_gama: GA registered by the maternity records (e3_gama). This is the obstetrician estimation, which is usually based on ultrasound measurements or LMP and possibly corrected for long durations by the obstetrician.

Child sex: (0 = female, 1 = male).

Child age at behavioral assessment: Continuous (years).

Maternal age: Continuous (years).

Maternal education: Maternal education was classified into 0 = low (primary or less), 1 = medium (secondary), 2 = high (university).

Child cognition (external variable): The total number of correct responses in Raven's Colored Progressive Matrices (Raven & Raven, 1998) was used as a measure of general cognitive index (continuous).

7. *INfancia y Medio Ambiente (INMA)*

Design and study population: The present study used data from participants recruited between 2003 and 2008 in the de novo cohort sited in Sabadell of the INfancia y Medio Ambiente (INMA; http://www.proyectoinma.org/) Project, a population-based mother–child cohort study in Spain (Guxens et al., 2012). Cord blood methylation was measured using the Infinium® HumanMethylation450 BeadChip. The number of participants with both DNA methylation and phenotype data is 292.

Consent and ethical approval: The study was approved by the Ethics Committee of the reference hospital, and all participants gave their written informed consent.

DNA methylation measurements: Cord blood was extracted using the Chemagen kit (Perkin Elmer). DNA concentration was determined by NanoDrop spectrophotometer (Thermo Scientific) and with the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies). Methylation data was produced in two different laboratories as part of two different projects: in the Genome Analysis Facility of the University Medical Center Groningen (UMCG) in Holland, and in the Bellvitge Biomedical Research Institute (IDIBELL, Barcelona). Both laboratories used the recommended Illumina protocol for the Infinium HumanMethylation450 beadchip. Briefly, 500 ng of DNA was bisulfite-converted using the EZ

96-DNA methylation kit following the manufacturer's standard protocol, and DNA methylation measured using the Illumina Infinium HumanMethylation450 beadchip.DNA methylation data were preprocessed using the minfi package (Aryee et al., 2014).

A series of steps were completed for quality control and data analysis. The first step was low quality sample removal. First, 2 samples with bad overall quality or with low detection p-value according to the output of the MethylAid package (van Iterson et al., 2014) were removed. Then, we removed 3 samples whose sex was wrongly predicted using shinyMethyl (Fortin et al., 2014). Following guidelines of Lehne work (Lehne et al., 2015), we increased the stringency of the detection p-value threshold to 10-16 and we filtered 18 samples with a call rate lower than 98%. The second step was normalizing data with functional normalization. Correlation between SNP in replicates samples was checked and probes not measuring SNPs were discarded. 7,136 probes with a call rate lower than 95% were also removed. Probes in sexual chromosomes, crosshibridizing or containing SNPs were flagged but not removed at this point. ComBat was applied to remove batch effect (Johnson et al., 2007). Finally, duplicated samples were removed. The final dataset consisted of 292 samples and 476,946 probes with behavioral data available.

Child psychopathology data: Psychopathology symptoms were assessed with the Child Behavior Checklist 6-18 (CBCL/6-18), a validated and widely used parental assessment of a child's behavioral and emotional problems (Achenbach & Rescorla, 2001). Mothers completed questions about a range of emotional and behavioral problems of the child in the past six months on a three-point scale (0=not true, 1=somewhat true, 2=very true).

Cell type correction: Cord blood reference panel with 7 cell types by Gervin et al. (2019).

Batch correction: ComBat was applied to remove batch effect (Johnson et al., 2007).

Ancestry/ethnicity: All individuals are classified as Europeans taking into account ethnic origin and country of origin of both parents

Smoking during pregnancy: Three-level ordinal category (never smoked during pregnancy, smoked during first trimester only, smoked after first trimester/sustained smoking).

Gestational age: Gestational age at blood sampling was calculated based on last menstrual period (LMP) reported at recruitment and confirmed using estimates based on ultrasound examina-tion in the 12th week of gestation. When the difference between the LMP reported at recruitment and estimated from the ultra-sound was ≥ 7 days (n=91; 16%), we esti-mated LMP using a quadratic regression formula (Westerway, Davison, & Cowell, 2000).

Child sex: (0 = female, 1 = male). Derived from medical/maternity records at birth

Child age at behavioral assessment: Continuous (years).

Maternal age: Continuous (years).

Maternal education: Maternal education was classified into 0 = low (primary or less), 1 = medium (secondary), 2 = high (university).

Child cognition (external variable): General cognitive index (continuous) was assessed using the McCarthy Scales of Children's Abilities (MCSA; McCarthy, 1996) at age 5 (mean=5.09; SD=0.69; Range=4.03-6.86).

References

Achenbach, T. M., & Rescorla, L. A. (2001). *Manual for the ASEBA School-Age Forms and Profiles*. Burlington, VT: University of Vermont Research Center for Children, Youth, & Families.

Alfano, R., Guida, F., Galobardes, B., Chadeau-Hyam, M., Delpierre, C., Ghantous, A., Henderson, J., Herceg, Z., Jain, P., Nawrot, T. S., Relton, C., Vineis, P., Castagné, R., & Plusquin, M. (2019). Socioeconomic position during pregnancy and DNA methylation signatures at three stages across early life: Epigenome-wide association studies in the ALSPAC birth cohort. International Journal of Epidemiology, 48(1), 30-44. https://doi.org/10.1093/ije/dyy259

Aryee, M. J., Jaffe, A. E., Corrada-Bravo, H., Ladd-Acosta, C., Feinberg, A. P., Hansen, K. D., & Irizarry, R. A. (2014). Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics, 30*(10), 1363-1369. doi:10.1093/bioinformatics/btu049

Ashford, J., van Lier, P. A. C., Timmermans, M., Cuijpers, P., & Koot, H. M. (2008). Prenatal smoking and internalizing and externalizing problems in children studied from childhood to late adolescence. Journal of the American Academy of Child and Adolescent Psychiatry, 47(7), 779-787. https://doi.org/10.1097/CHI.0b013e318172eefb

Boyd, A., Golding, J., Macleod, J., Lawlor, D. A., Fraser, A., Henderson, J., . . . Davey Smith, G. (2013). Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol, 42*(1), 111-127. doi:10.1093/ije/dys064

Carslake, D., Tynelius, P., van den Berg, G., Davey Smith, G., & Rasmussen, F. (2017). Associations of parental age with health and social factors in adult offspring. Methodological pitfalls and possibilities. Scientific Reports, 7, 45278. https://doi.org/10.1038/srep45278

Chen, Y. A., Lemire, M., Choufani, S., Butcher, D. T., Grafodatskaya, D., Zanke, B. W., . . . Weksberg, R. (2013). Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. *Epigenetics, 8*(2), 203-209. doi:10.4161/epi.23470

Chu, S.-K., & Yang, H.-C. (2017). Interethnic DNA methylation difference and its implications in pharmacoepigenetics. Epigenomics, 9(11), 1437-1454. https://doi.org/10.2217/epi-2017-0046

Cosin-Tomas, M., Cilleros-Portet, A., Aguilar-Lacasaña, S., Fernandez-Jimenez, N., & Bustamante, M. (2022). Prenatal Maternal Smoke, DNA Methylation, and Multi-omics of Tissues and Child Health. Current Environmental Health Reports, 9(3), 502-512. https://doi.org/10.1007/s40572-022-00361-9

Du, P., Kibbe, W. A., & Lin, S. M. (2008). lumi: a pipeline for processing Illumina microarray. *Bioinformatics, 24*(13), 1547-1548. doi:btn224 [pii]

10.1093/bioinformatics/btn224

Fortin, J. P., Labbe, A., Lemire, M., Zanke, B. W., Hudson, T. J., Fertig, E. J., . . . Hansen, K. D. (2014). Functional normalization of 450k methylation array data improves replication in large cancer studies. *Genome Biol, 15*(12), 503. doi:10.1186/s13059-014-0503-2

Fraser, A., Macdonald-Wallis, C., Tilling, K., Boyd, A., Golding, J., Davey Smith, G., . . . Lawlor, D. A. (2013). Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. *Int J Epidemiol, 42*(1), 97-110. doi:10.1093/ije/dys066

Gaunt, T. R., Shihab, H. A., Hemani, G., Min, J. L., Woodward, G., Lyttleton, O., . . . Relton, C. L. (2016). Systematic identification of genetic influences on methylation across the human life course. *Genome Biol, 17*, 61. doi:10.1186/s13059-016-0926-z

Gervin, K., Salas, L. A., Bakulski, K. M., van Zelm, M. C., Koestler, D. C., Wiencke, J. K., . . . Jones, M. J. (2019). Systematic evaluation and validation of reference and library selection methods for deconvolution of cord blood DNA methylation data. *Clin Epigenetics, 11*(1), 125. doi:10.1186/s13148-019-0717-y

Goodman, A., Heiervang, E., Collishaw, S., & Goodman, R. (2011). The 'DAWBA bands' as an ordered-categorical measure of child mental health: description and validation in British and Norwegian samples. *Soc Psychiatry Psychiatr Epidemiol, 46*(6), 521-532. doi:10.1007/s00127-010-0219-x

Goodman, R., Ford, T., Richards, H., Gatward, R., & Meltzer, H. (2000). The Development and Well-Being Assessment: description and initial validation of an integrated assessment of child and adolescent psychopathology. *J Child Psychol Psychiatry, 41*(5), 645-655.

Guxens, M., Ballester, F., Espada, M., Fernandez, M. F., Grimalt, J. O., Ibarluzea, J., . . . Project, I. (2012). Cohort Profile: the INMA--INfancia y Medio Ambiente--(Environment and Childhood) Project. *Int J Epidemiol, 41*(4), 930-940. doi:10.1093/ije/dyr054

Hannum, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S., . . . Zhang, K. (2013). Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell, 49*(2), 359-367. doi:10.1016/j.molcel.2012.10.016

Houseman, E. A., Accomando, W. P., Koestler, D. C., Christensen, B. C., Marsit, C. J., Nelson, H. H., . . . Kelsey, K. T. (2012). DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics, 13*, 86. doi:10.1186/1471-2105-13-86

Jaffe, A. E., & Irizarry, R. A. (2014). Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome Biol, 15*(2), R31. doi:10.1186/gb-2014-15-2-r31

Johnson, W. E., Li, C., & Rabinovic, A. (2007). Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics, 8*(1), 118-127. doi:10.1093/biostatistics/kxj037

Kooijman, M. N., Kruithof, C. J., van Duijn, C. M., Duijts, L., Franco, O. H., van, I. M. H., . . . Jaddoe, V. W. (2016). The Generation R Study: design and cohort update 2017. *Eur J Epidemiol, 31*(12), 1243-1264. doi:10.1007/s10654-016-0224-9

Leek, J. T., Johnson, W. E., Parker, H. S., Fertig, E. J., Jaffe, A. E., Storey, J. D., . . . Torres, L. C. (2019). sva: Surrogate Variable Analysis. doi:10.1093/bioinformatics/bts034

Lehne, B., Drong, A. W., Loh, M., Zhang, W., Scott, W. R., Tan, S. T., . . . Chambers, J. C. (2015). A coherent approach for analysis of the Illumina HumanMethylation450 BeadChip improves data quality and performance in epigenome-wide association studies. *Genome Biol, 16*, 37. doi:10.1186/s13059-015-0600-x

Magnus, P., Birke, C., Vejrup, K., Haugan, A., Alsaker, E., Daltveit, A. K., . . . Stoltenberg, C. (2016). Cohort Profile Update: The Norwegian Mother and Child Cohort Study (MoBa). *Int J Epidemiol, 45*(2), 382-388. doi:10.1093/ije/dyw029

Maitre, L., de Bont, J., Casas, M., Robinson, O., Aasvang, G. M., Agier, L., . . . Vrijheid, M. (2018). Human Early Life Exposome (HELIX) study: a European population-based exposome cohort. *BMJ Open, 8*(9), e021311. doi:10.1136/bmjopen-2017-021311

Markunas, C. A., Wilcox, A. J., Xu, Z., Joubert, B. R., Harlid, S., Panduri, V., Håberg, S. E., Nystad, W., London, S. J., Sandler, D. P., Lie, R. T., Wade, P. A., & Taylor, J. A. (2016). Maternal Age at Delivery Is Associated with an Epigenetic Signature in Both Newborns and Adults. PloS One, 11(7), e0156361. https://doi.org/10.1371/journal.pone.0156361

McCarthy, D. (1996). *Manual for the McCarthy Scales of Children's Abilities, Spanish version*. Madrid, Spain: TEA Ediciones, S.A.

McCartney, D. L., Walker, R. M., Morris, S. W., McIntosh, A. M., Porteous, D. J., & Evans, K. L. (2016). Identification of polymorphic and off-target probe binding sites on the Illumina Infinium MethylationEPIC BeadChip. *Genom Data, 9*, 22-24. doi:10.1016/j.gdata.2016.05.012

Merid, S. K., Novoloaca, A., Sharp, G. C., Küpers, L. K., Kho, A. T., Roy, R., Gao, L., Annesi-Maesano, I., Jain, P., Plusquin, M., Kogevinas, M., Allard, C., Vehmeijer, F. O., Kazmi, N., Salas, L. A., Rezwan, F. I., Zhang, H., Sebert, S., Czamara, D., … Melén, E. (2020). Epigenome-wide

meta-analysis of blood DNA methylation in newborns and children identifies numerous loci related to gestational age. Genome Medicine, 12(1), 25. https://doi.org/10.1186/s13073-020-0716-9

Meyrose, A.-K., Klasen, F., Otto, C., Gniewosz, G., Lampert, T., & Ravens-Sieberer, U. (2018). Benefits of maternal education for mental health trajectories across childhood and adolescence. Social Science & Medicine (1982), 202, 170-178. https://doi.org/10.1016/j.socscimed.2018.02.026

Min, J. L., Hemani, G., Davey Smith, G., Relton, C., & Suderman, M. (2018). Meffil: efficient normalization and analysis of very large DNA methylation datasets. *Bioinformatics, 34*(23), 3983-3989. doi:10.1093/bioinformatics/bty476

Morin, A. M., Gatev, E., McEwen, L. M., MacIsaac, J. L., Lin, D. T. S., Koen, N., . . . Jones, M. J. (2017). Maternal blood contamination of collected cord blood can be identified using DNA methylation at three CpGs. *Clin Epigenetics, 9*, 75. doi:10.1186/s13148-017-0370-2

Moster, D., Lie, R. T., & Markestad, T. (2008). Long-term medical and social consequences of preterm birth. The New England Journal of Medicine, 359(3), 262-273. https://doi.org/10.1056/NEJMoa0706475

Mulder, R. H., Neumann, A., Cecil, C. A. M., Walton, E., Houtepen, L. C., Simpkin, A. J., Rijlaarsdam, J., Heijmans, B. T., Gaunt, T. R., Felix, J. F., Jaddoe, V. W. V., Bakermans-Kranenburg, M. J., Tiemeier, H., Relton, C. L., van IJzendoorn, M. H., & Suderman, M. (2021). Epigenome-wide change and variation in DNA methylation in childhood: Trajectories from birth to late adolescence. Human Molecular Genetics, 30(1), 119-134. https://doi.org/10.1093/hmg/ddaa280

Northstone, K., Lewcock, M., Groom, A., Boyd, A., Macleod, J., Timpson, N., & Wells, N. (2019). The Avon Longitudinal Study of Parents and Children (ALSPAC): an update on the enrolled sample of index children in 2019. *Wellcome Open Res, 4*, 51. doi:10.12688/wellcomeopenres.15132.1

Pidsley, R., CC, Y. W., Volta, M., Lunnon, K., Mill, J., & Schalkwyk, L. C. (2013). A data-driven approach to preprocessing Illumina 450K methylation array data. *BMC Genomics, 14*, 293. doi:10.1186/1471-2164-14-293

Price, M. E., Cotton, A. M., Lam, L. L., Farre, P., Emberly, E., Brown, C. J., . . . Kobor, M. S. (2013). Additional annotation enhances potential for biologically-relevant analysis of the Illumina Infinium HumanMethylation450 BeadChip array. *Epigenetics Chromatin, 6*(1), 4. doi:10.1186/1756-8935-6-4

Raven, J. C., & Raven, J. H. (1998). *Raven coloured progressive matrices manual*. San Antonio. Texas. USA: Harcourt assessment.

Reinius, L. E., Acevedo, N., Joerink, M., Pershagen, G., Dahlen, S. E., Greco, D., . . . Kere, J. (2012). Differential DNA methylation in purified human blood cells: implications for cell lineage and studies on disease susceptibility. *PLoS One, 7*(7), e41361. doi:10.1371/journal.pone.0041361

Relton, C. L., Gaunt, T., McArdle, W., Ho, K., Duggirala, A., Shihab, H., . . . Davey Smith, G. (2015). Data Resource Profile: Accessible Resource for Integrated Epigenomic Studies (ARIES). *Int J Epidemiol, 44*(4), 1181-1190. doi:10.1093/ije/dyv072

Riecher-Rössler, A. (2017). Sex and gender differences in mental disorders. The Lancet. Psychiatry, 4(1), 8-9. https://doi.org/10.1016/S2215-0366(16)30348-0

Salas, L. A., Gervin, K., & Jones, M. C. (2020). FlowSorted.CordBloodCombined.450k: Illumina 450k/EPIC data on FACS and MACS umbilical blood cells. R package version 1.7.0, https://github.com/immunomethylomics/FlowSorted.CordBloodCombined.450k.

Solmi, M., Radua, J., Olivola, M., Croce, E., Soardo, L., Salazar de Pablo, G., Il Shin, J., Kirkbride, J. B., Jones, P., Kim, J. H., Kim, J. Y., Carvalho, A. F., Seeman, M. V., Correll, C. U., & Fusar-Poli, P. (2022). Age at onset of mental disorders worldwide: Large-scale meta-analysis of 192 epidemiological studies. Molecular Psychiatry, 27(1), 281-295. https://doi.org/10.1038/s41380-021-01161-7

Strandberg, T. E., Jarvenpaa, A. L., Vanhanen, H., & McKeigue, P. M. (2001). Birth outcome in relation to licorice consumption during pregnancy. *Am J Epidemiol, 153*(11), 1085-1088. doi:10.1093/aje/153.11.1085

Suderman, M., Hemani, G., & Min, J. (2019). meffil: Efficient algorithms for DNA methylation. https://github.com/perishky/meffil.

Tellegen, P., Winkel, M., Wijnberg-Williams, B., & Laros, J. A. (2005). *Snijders-Oomen niet-verbale intelligentietest: SON-R 2½–7*. Amsterdam, The Netherlands: Boom Test Uitgevers.

Triche, T. J., Jr., Weisenberger, D. J., Van Den Berg, D., Laird, P. W., & Siegmund, K. D. (2013). Low-level processing of Illumina Infinium DNA Methylation BeadArrays. *Nucleic Acids Res, 41*(7), e90. doi:10.1093/nar/gkt090

van Iterson, M., Tobi, E. W., Slieker, R. C., den Hollander, W., Luijk, R., Slagboom, P. E., & Heijmans, B. T. (2014). MethylAid: visual and interactive quality control of large Illumina 450k datasets. *Bioinformatics, 30*(23), 3435-3437. doi:10.1093/bioinformatics/btu566

Wechsler, D., Golombok, S., & Rust, J. (1992). *WISC-III UK Wechsler intelligence scale for children: UK manual*. Sidcup, UK: The Psychological Corporation.

Westerway, S. C., Davison, A., & Cowell, S. (2000). Ultrasonic fetal measurements: new Australian standards for the new millennium. *Aust N Z J Obstet Gynaecol, 40*(3), 297-302. doi:10.1111/j.1479-828x.2000.tb03338.x

Yousefi, P., Huen, K., Davé, V., Barcellos, L., Eskenazi, B., & Holland, N. (2015). Sex differences in DNA methylation assessed by 450 K BeadChip in newborns. BMC Genomics, 16, 911. https://doi.org/10.1186/s12864-015-2034-y

Zar, H. J., Barnett, W., Myer, L., Stein, D. J., & Nicol, M. P. (2015). Investigating the early-life determinants of illness in Africa: the Drakenstein Child Health Study. *Thorax, 70*(6), 592-594. doi:10.1136/thoraxjnl-2014-206242