

1 **Identification of autosomal cis expression**  
2 **quantitative trait methylation (cis eQTM) in**  
3 **children's blood**

4 Carlos Ruiz-Arenas<sup>1,2</sup>, Carles Hernandez-Ferrer<sup>2,3,4</sup>, Marta Vives-Usano<sup>2,4,5</sup>, Sergi Mari<sup>2,4,6</sup>,  
5 Inés Quintela<sup>7</sup>, Dan Mason<sup>8</sup>, Solène Cadiou<sup>9</sup>, Maribel Casas<sup>2,4</sup>, Sandra Andrusaityte<sup>10</sup>,  
6 Kristine Bjerve Gutzkow<sup>11</sup>, Marina Vafeiadi<sup>12</sup>, John Wright<sup>8</sup>, Johanna Lepeule<sup>9</sup>, Regina  
7 Grazuleviciene<sup>10</sup>, Leda Chatzi<sup>13</sup>, Ángel Carracedo<sup>14,15</sup>, Xavier Estivill<sup>16</sup>, Eulàlia Martí<sup>6,17</sup>,  
8 Geòrgia Escaramís<sup>6,17</sup>, Martine Vrijheid<sup>2,4,6</sup>, Juan R González<sup>2,4,6\*</sup>, Mariona Bustamante<sup>2,4,6\*</sup>

9 Affiliations:

10 1. Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER),  
11 Barcelona, Spain

12 2. Universitat Pompeu Fabra (UPF), Barcelona, Spain

13 3. Centro Nacional de Análisis Genómico (CNAG-CRG), Center for Genomic Regulation,  
14 Barcelona Institute of Science and Technology (BIST), Barcelona, Catalonia, Spain.

15 4. ISGlobal, Dr Aiguader 88, 08003 Barcelona, Spain

16 5. Center for Genomic Regulation (CRG), Barcelona Institute of Science and Technology, Av  
17 Aiguader 88, 08003 Barcelona, Spain

18 6. CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain

19 7. Medicine Genomics Group, University of Santiago de Compostela, CEGEN-PRB3, 15782,  
20 Santiago de Compostela, Spain

21 8. Bradford Institute for Health Research, Bradford Teaching Hospitals NHS Foundation  
22 Trust, UK

23 9. University Grenoble Alpes, Inserm, CNRS, Team of Environmental Epidemiology Applied  
24 to Reproduction and Respiratory Health, IAB, 38000 Grenoble, France

25 10. Department of Environmental Science, Vytautas Magnus University, 44248 Kaunas,  
26 Lithuania

27 11. Department of Environmental Health, Norwegian Institute of Public Health, Oslo, Norway

28 12. Department of Social Medicine, University of Crete, Greece

29 13. Department of Preventive Medicine, Keck School of Medicine, University of Southern  
30 California, USA

31 14. Medicine Genomics Group, CIBERER, University of Santiago de Compostela, CEGEN-  
32 PRB3, 15782, Santiago de Compostela, Spain

33 15. Galician Foundation of Genomic Medicine, IDIS, SERGAS, 15706, Santiago de  
34 Compostela, Spain

35 16. Quantitative Genomics Medicine Laboratories (qGenomics), Esplugues del Llobregat,  
36 Barcelona, Catalonia, Spain.

37 17. Departament de Biomedicina, Institut de Neurociències, Universitat de Barcelona,  
38 Barcelona, Spain

39

40 **Corresponding authors:**

41 Carlos Ruiz-Arenas: carlos.ruiza@upf.edu

42 Mariona Bustamante: mariona.bustamante@isglobal.org

43

## 44 **Abstract**

45 **Background:** The identification of expression quantitative trait methylation (eQTM), defined  
46 as associations between DNA methylation levels and gene expression, might help the  
47 biological interpretation of epigenome-wide association studies (EWAS). We aimed to  
48 identify autosomal cis eQTMs in children's blood, using data from 832 children of the Human  
49 Early Life Exposome (HELIX) project.

50 **Methods:** Blood DNA methylation and gene expression were measured with the Illumina  
51 450K and the Affymetrix HTA v2 arrays, respectively. The relationship between methylation  
52 levels and expression of nearby genes (1 Mb window centered at the transcription start site,  
53 TSS) was assessed by fitting 13.6 M linear regressions adjusting for sex, age, cohort, and  
54 blood cell composition.

55 **Results:** We identified 39,749 blood autosomal cis eQTMs, representing 21,966 unique  
56 CpGs (eCpGs, 5.7% of total CpGs) and 8,886 unique transcript clusters (eGenes, 15.3% of  
57 total transcript clusters, equivalent to genes). In 87.9% of these cis eQTMs, the eCpG was  
58 located at <250 kb from eGene's TSS; and 58.8% of all eQTMs showed an inverse  
59 relationship between the methylation and expression levels. Only around half of the  
60 autosomal cis-eQTMs eGenes could be captured through annotation of the eCpG to the  
61 closest gene. eCpGs had less measurement error and were enriched for active blood  
62 regulatory regions and for CpGs reported to be associated with environmental exposures or  
63 phenotypic traits. 40.4% of eQTMs had at least one genetic variant associated with  
64 methylation and expression levels. The overlap of autosomal cis eQTMs in children's blood  
65 with those described in adults was small (13.8%), and age-shared cis eQTMs tended to be  
66 proximal to the TSS and enriched for genetic variants.

67 **Conclusions:** This catalogue of autosomal cis eQTM in children's blood can help the  
68 biological interpretation of EWAS findings and is publicly available at  
69 <https://helixomics.isglobal.org/>.

70 **Funding:** The study has received funding from the European Community's Seventh  
71 Framework Programme (FP7/2007-2013) under grant agreement no 308333 (HELIX project);  
72 the H2020-EU.3.1.2. - Preventing Disease Programme under grant agreement no 874583  
73 (ATHLETE project); from the European Union's Horizon 2020 research and innovation  
74 programme under grant agreement no 733206 (LIFECYCLE project), and from the European  
75 Joint Programming Initiative "A Healthy Diet for a Healthy Life" (JPI HDHL and Instituto de  
76 Salud Carlos III) under the grant agreement no AC18/00006 (NutriPROGRAM project). The  
77 genotyping was supported by the project PI17/01225, funded by the Instituto de Salud  
78 Carlos III and co-funded by European Union (ERDF, "A way to make Europe") and the  
79 Centro Nacional de Genotipado-CEGEN (PRB2-ISCI).

80

## 81 **Keywords**

82 eQTM, quantitative trait, epigenetics, DNA methylation, transcription, gene expression,  
83 blood, children, EWAS

## 84 **Abbreviations**

85 BivFlnx: flanking bivalent region

86 CpG: cytosine nucleotide followed by a guanine nucleotide

87 eCpG: CpG whose methylation is associated with gene expression; thus, it is part of an

88 eQTM

89 eGene: gene whose expression is associated with CpG methylation; thus, it is part of an

90 eQTM

91 eQTM: expression quantitative trait methylation (statistically significant associations of CpG-

92 gene pairs)

93 eQTL: expression quantitative trait locus (SNP associated with gene expression)

94 Enh: enhancer

95 EnhBiv: bivalent enhancer

96 EnhG: genic enhancer

97 EWAS: epigenome-wide association study

98 FC: fold change

99 FDR: false discovery rate

100 GO: gene ontology

101 GWAS: genome-wide association study

102 HELIX: Human Early-Life Exposome project

103 Het: heterochromatin

104 ICC: intraclass correlation coefficient

- 105 IQR: interquartile range
- 106 meQTL: methylation quantitative trait locus (SNP associated with DNA methylation)
- 107 OR: odds ratio
- 108 Quies: quiescent region
- 109 ReprPC: repressed Polycomb
- 110 ReprPCWk: weak repressed Polycomb
- 111 SE: standard error
- 112 SNP: single nucleotide polymorphism
- 113 TC: transcript cluster
- 114 TSS: transcription start site
- 115 TssA: active transcription start site
- 116 TssAFlnk: flanking active transcription start site
- 117 TssBiv: bivalent transcription start site
- 118 TSS200: proximal promoter, from TSS to 200 bp
- 119 TSS1500: distal promoter, from 200 bp to 1,500 bp
- 120 Tx: transcription region
- 121 TxFlnk: transcription at 5' and 3'
- 122 TxWk: weak transcription region
- 123 3'UTR: 3' untranslated region
- 124 5'UTR: 5' untranslated region

125 ZNF.Rpts: zinc finger genes and repeats

## 126 **Introduction**

127 Cells from the same individual, although sharing the same genome sequence, differentiate  
128 into diverse lineages that finally give place to specific cell types with unique functions. This is  
129 orchestrated by the epigenome, which regulates gene expression in a cell/tissue- and time-  
130 specific manner (Cavalli and Heard, 2019; Feinberg, 2018; Lappalainen and Grealley, 2017).  
131 Besides its central role in regulating embryonic and fetal development, X-chromosome  
132 inactivation, genomic imprinting, and silencing of repetitive DNA elements, the epigenome is  
133 also responsible for the plasticity and cellular memory in response to environmental  
134 perturbations (Cavalli and Heard, 2019; Feinberg, 2018; Lappalainen and Grealley, 2017).

135 Massive epigenetic alterations, caused by somatic mutations, age, injury, or environmental  
136 exposures, were initially described in cancer (Feinberg, 2018). The paradigm of  
137 environmental factors modifying the epigenome and leading to increased disease risk was  
138 then extrapolated from cancer to a wide range of common diseases. Consequently, in recent  
139 years, a high number of epigenome-wide association studies (EWAS) have been performed,  
140 investigating the relation of prenatal and postnatal exposure to environmental factors with  
141 DNA methylation, and of DNA methylation with disease (Feinberg, 2018; Lappalainen and  
142 Grealley, 2017). EWAS findings have been inventoried in two catalogues: the EWAS catalog  
143 (Batram et al., 2021) and the EWAS Atlas (Li et al., 2019). The latter includes 0.5 M  
144 associations for 498 traits from 1,216 studies, including 155 different cells/tissues.

145 Despite the success of EWAS in identifying altered methylation patterns, various challenging  
146 issues still must be solved: the role of genetic variation; the access to the target tissue/cell;  
147 confounding reverse causation; and biological interpretation (Feinberg, 2018; Lappalainen  
148 and Grealley, 2017). Regarding the latter, most studies do not have transcriptional data to test  
149 the effect of DNA methylation on gene expression. When these data are not available, a

150 common approach is to assume that CpG DNA methylation affects the expression of the  
151 closest gene (Sharp et al., 2017). Although this approach is easy to implement, it is limited.  
152 Indeed, CpG DNA methylation might regulate distant genes or might not regulate any gene  
153 at all (Bonder et al., 2017; Lappalainen and Greally, 2017). Another approach to elucidate  
154 the effect of DNA methylation on gene expression when transcriptional data are not available  
155 is to perform expression quantitative trait methylation (eQTM) studies. These are genome-  
156 wide studies investigating the associations between the levels of DNA methylation and gene  
157 expression (Gondalia et al., 2019; Küpers et al., 2019). Several eQTM studies have been  
158 performed in diverse cell types/tissues: whole blood (Bonder et al., 2017; Kennedy et al.,  
159 2018), monocytes (Husquin et al., 2018; Kennedy et al., 2018; Liu et al., 2013),  
160 lymphoblastoid cell lines, T-cells and fibroblasts derived from umbilical cords (Gutierrez-  
161 Arcelus et al., 2015, 2013), fibroblasts (Wagner et al., 2014), liver (Bonder et al., 2014),  
162 skeletal muscle (Leland Taylor et al., 2019), nasal airway epithelium (Kim et al., 2020), and  
163 placenta (Delahaye et al., 2018). As most of the EWAS are conducted in whole blood (Felix  
164 et al., 2018; Li et al., 2019), there is a need for comprehensive eQTM studies in this tissue.  
165 To date, available eQTM studies in whole blood only cover samples from adults (Bonder et  
166 al., 2017; Kennedy et al., 2018) and their validity in children has not been assessed.

167 In this study, we analyzed DNA methylation and gene expression data from the Human  
168 Early-Life Exposome (HELIX) project to an autosomal cis eQTM catalogue in children's  
169 blood (<https://helixomics.isglobal.org/>). We analyzed the proportion of cis eQTMs captured  
170 through annotation to the closest gene, characterized them at the functional level, assessed  
171 the influence of genetic variation and compared them with eQTMs identified in adults. An  
172 overview of all the analyses can be found in Figure 1. This public resource will help the  
173 functional interpretation of EWAS findings in children.



## 174 **Methods**

### 175 **Sample of the study**

176 The Human Early Life Exposome (HELIX) study is a collaborative project across 6  
177 established and on-going longitudinal population-based birth cohort studies in Europe  
178 (Maitre et al., 2018): the Born in Bradford (BiB) study in the UK (Wright et al., 2013), the  
179 Étude des Déterminants pré et postnatals du développement et de la santé de l'Enfant  
180 (EDEN) study in France (Heude et al., 2016), the Infancia y Medio Ambiente (INMA) cohort  
181 in Spain (Guxens et al., 2012), the Kaunus cohort (KANC) in Lithuania (Grazuleviciene et al.,  
182 2009), the Norwegian Mother, Father and Child Cohort Study (MoBa)(Magnus et al., 2016)  
183 and the RHEA Mother Child Cohort study in Crete, Greece (Chatzi et al., 2017). All  
184 participants in the study signed an ethical consent and the study was approved by the ethical  
185 committees of each study area (Maitre et al., 2018).

186 In the present study, we selected a total of 832 children of European ancestry that had both  
187 DNA methylation and gene expression data. Ancestry was determined with cohort-specific  
188 self-reported questionnaires.

### 189 **Biological samples**

190 DNA was obtained from buffy coats collected in EDTA tubes at mean age 8.1 years old.  
191 Briefly, DNA was extracted using the Chemagen kit (Perkin Elmer), in batches by cohort.  
192 DNA concentration was determined in a NanoDrop 1000 UV-Vis Spectrophotometer  
193 (Thermo Fisher Scientific) and with Quant-iT™ PicoGreen® dsDNA Assay Kit (Life  
194 Technologies).

195 RNA was extracted from whole blood samples collected in Tempus tubes (Applied  
196 Biosystems) using the MagMAX for Stabilized Blood Tubes RNA Isolation Kit (Thermo

197 Fisher Scientific), in batches by cohort. The quality of RNA was evaluated with a 2100  
198 Bioanalyzer (Agilent) and the concentration with a NanoDrop 1000 UV-Vis  
199 Spectrophotometer (Thermo Fisher Scientific). Samples classified as good RNA quality had  
200 an RNA Integrity Number (RIN) > 5, a similar RNA integrity pattern at visual inspection, and  
201 a concentration >10 ng/ul. Mean values for the RIN, concentration (ng/ul) and Nanodrop  
202 260/230 ratio were: 7.05, 109.07 and 2.15, respectively.

## 203 DNA methylation assessment

204 DNA methylation was assessed with the Infinium HumanMethylation450K BeadChip  
205 (Illumina), following manufacturer's protocol at the National Spanish Genotyping Centre  
206 (CEGEN), Spain. Briefly, 700 ng of DNA were bisulfite-converted using the EZ 96-DNA  
207 methylation kit following the manufacturer's standard protocol, and DNA methylation  
208 measured using the Infinium protocol. A HapMap sample was included in each plate. In  
209 addition, 24 HELIX inter-plate duplicates were included. Samples were randomized  
210 considering cohort, sex, and panel. Paired samples from the panel study (samples from the  
211 same subject collected at different time points) were processed in the same array. Two  
212 samples were repeated due to their overall low quality.

213 DNA methylation data was pre-processed using *minfi* R package (RRID:SCR\_012830)  
214 (Aryee et al., 2014). We increased the stringency of the detection p-value threshold to <1e-  
215 16, and probes not reaching a 98% call rate were excluded (Lehne et al., 2015). Two  
216 samples were filtered due to overall quality: one had a call rate <98% and the other did not  
217 pass quality control parameters of the *MethylAid* R package (RRID:SCR\_002659) (van  
218 Iterson et al., 2014). Then, data was normalized with the functional normalization method  
219 with Noob background subtraction and dye-bias correction (Fortin et al., 2014b). Then, we  
220 checked sex consistency using the *shinyMethyl* R package (Fortin et al., 2014a), genetic  
221 consistency of technical duplicates, biological duplicates (panel study), and other samples  
222 making use of the genotype probes included in the Infinium HumanMethylation450K

223 BeadChip and the genome-wide genotyping data, when available. In total four samples were  
224 excluded, two with discordant sex and two with discordant genotypes. Batch effect (slide)  
225 was corrected using the *ComBat* R package (RRID:SCR\_010974) (Johnson et al., 2007).  
226 Duplicated samples, one of the samples from the panel study and HapMap samples were  
227 removed as well as control probes, probes in sexual chromosomes, probes designed to  
228 detect Single Nucleotide Polymorphisms (SNPs) and probes to measure methylation levels  
229 at non-CpG sites, giving a final number of 386,518 probes.

230 CpG annotation was conducted with the *IlluminaHumanMethylation450kanno.ilmn-12.hg19*  
231 R package (Hansen, n.d.). Briefly, this package annotates CpGs to proximal promoter (200  
232 bp upstream the TSS - TSS200), distant promoter (from 200 to 1,500 bp upstream the TSS -  
233 TSS1500), 5'UTR, first exon, gene body, and 3'UTR regions. CpGs farther than 1,500 bp  
234 from the TSS were not annotated to any gene. Relative position to CpG islands (island,  
235 shelve, shore and open sea) was also provided by the same R package.

236 Annotation of CpGs to 15 chromatin states was retrieved from the Roadmap Epigenomics  
237 Project web portal (RRID:SCR\_008924) ([https://egg2.wustl.edu/roadmap/web\\_portal/](https://egg2.wustl.edu/roadmap/web_portal/)). Each  
238 CpG in the array was annotated to one or several chromatin states by taking a state as  
239 present in that locus if it was described in at least 1 of the 27 blood-related cell types.

## 240 Gene expression assessment

241 Gene expression, including coding and non-coding transcripts, was assessed with the  
242 Human Transcriptome Array 2.0 ST arrays (HTA 2.0) (Affymetrix) at the University of  
243 Santiago de Compostela (USC), Spain. Amplified and biotinylated sense-strand DNA targets  
244 were generated from total RNA. Affymetrix HTA 2.0 arrays were hybridized according to  
245 Affymetrix recommendations using the Manual Target preparation for GeneChip Whole  
246 Transcript (WT) expression arrays and the labeling and hybridization kits. In each round,  
247 several batches of 24-48 samples were processed. Samples were randomized within each

248 batch considering sex and cohort. Paired samples from the panel study were processed in  
249 the same batch. Two different types of control RNA samples (HeLa or FirstChoice® Human  
250 Brain Reference RNA) were included in each batch, but they were hybridized only in the first  
251 batches. Raw data were extracted with the AGCC software (Affymetrix) and stored into CEL  
252 files. Ten samples failed during the laboratory process (7 did not have enough cRNA or ss-  
253 cDNA, 2 had low fluorescence, and 1 presented an artifact in the CEL file).

254 Data was normalized with the GCCN (SST-RMA) algorithm at the gene level. Annotation of  
255 transcript clusters (TCs) was done with the ExpressionConsole software using the HTA-2.0  
256 Transcript Cluster Annotations Release na36 annotation file from Affymetrix. After  
257 normalization, several quality control checks were performed and four samples with  
258 discordant sex and two with low call rates were excluded (Buckberry et al., 2014). One of the  
259 samples from the panel study was also eliminated for this analysis. Control probes and  
260 probes in sexual chromosomes or probes without chromosome information were excluded.  
261 Probes with a DABG (Detected Above Background) p-value <0.05 were considered to have  
262 an expression level different from the background, and they were defined as detected.  
263 Probes with a call rate <1% were excluded from the analysis. The final dataset consisted of  
264 58,254 TCs.

265 Gene expression values were  $\log_2$  transformed and batch effect controlled by residualizing  
266 the effect of surrogate variables calculated with the sva method (RRID:SCR\_012836) (Leek  
267 et al., 2007) while protecting for main variables in the study (cohort, age, sex, and blood  
268 cellular composition).

## 269 Blood cellular composition

270 Main blood cell type proportions (CD4+ and CD8+ T-cells, natural killer cells, monocytes,  
271 eosinophils, neutrophils, and B-cells) were estimated using the Houseman algorithm

272 (Houseman et al., 2012) and the Reinius reference panel (Reinius et al., 2012) from raw  
273 methylation data.

## 274 Genome-wide genotyping

275 Genome-wide genotyping was performed using the Infinium Global Screening Array (GSA)  
276 MD version 1 (Illumina), which contains 692,367 variants, at the Human Genomics Facility  
277 (HuGe-F), Erasmus MC, The Netherlands. Genotype calling was done using the  
278 GenTrain2.0 algorithm based on a custom cluster file implemented in the GenomeStudio  
279 software (RRID:SCR\_010973). Annotation was done with the GSAMD-24v1-  
280 0\_20011747\_A4 manifest. Samples were genotyped in two rounds, and 10 duplicates were  
281 included which confirmed high inter-round consistency.

282 Quality control was performed with the PLINK program (RRID:SCR\_001757) following  
283 standard recommendations (Chang et al., 2015; Purcell et al., 2007). We applied the  
284 following sample quality controls: sample call rate <97% (N filtered=43), sex concordance  
285 (N=8), heterozygosity based on >4 SD (N=0), relatedness with PI\_HAT >0.185 (N=10,  
286 including potential DNA contamination), duplicates (N=19). Then, we used the *peddy* tool  
287 (RRID:SCR\_017287) to predict ancestry from GWAS data (Pedersen and Quinlan, 2017).  
288 We contrasted ancestry predicted from GWAS with ancestry recorded in the questionnaires.  
289 Twelve samples were excluded due to discordances between the two variables. Overall, 93  
290 (6.7%) samples, including the duplicates, were filtered out. The variant quality control  
291 included the following steps: variant call rate <95% (N filtered=4,046), non-canonical PAR  
292 (N=47), minor allele frequency (MAF) <1% (N=178,017), Hardy-Weinberg equilibrium (HWE)  
293 p-value <1e-06 (N=913). Some other SNPs were filtered out during the matching between  
294 data and reference panel before imputation (N=14,436).

295 Imputation of the GWAS data was performed with the Imputation Michigan server  
296 (RRID:SCR\_017579) (Das et al., 2016) using the Haplotype Reference Consortium (HRC)

297 cosmopolitan panel, Version r1.1 2016 (McCarthy et al., 2016). Before imputation, PLINK  
298 GWAS data was converted into VCF format and variants were aligned with the reference  
299 genome. The phasing of the haplotypes was done with Eagle v2.4 (RRID:SCR\_017262)  
300 (Loh et al., 2016) and the imputation with minimac4 (RRID:SCR\_009292) (Fuchsberger et  
301 al., 2015), both implemented in the code of the Imputation Michigan server. In total, we  
302 retrieved 40,405,505 variants after imputation. Then, we applied the following QC criteria to  
303 the imputed dataset: imputation accuracy ( $R^2$ )  $>0.9$ , MAF  $>1\%$ , HWE p-value  $>1e-06$ ; and  
304 genotype probabilities were converted to genotypes using the best guess approach. The final  
305 post-imputation quality-controlled dataset consisted of 1,304 samples and 6,143,757  
306 variants (PLINK format, Genome build: GRCh37/hg19, + strand).

## 307 Identification of autosomal cis eQTM in children's blood

308 To test associations between DNA methylation levels and gene expression levels in cis (cis  
309 eQTMs), we paired each Gene to CpGs closer than 500 kb from its TSS, either upstream or  
310 downstream. For each Gene, the TSS was defined based on HTA-2.0 annotation, using the  
311 start position for transcripts in the + strand, and the end position for transcripts in the -  
312 strand. CpGs position was obtained from Illumina 450K array annotation. Only CpGs in  
313 autosomal chromosomes (from chromosome 1 to 22) were tested. In the main analysis, we  
314 fitted for each CpG-Gene pair a linear regression model between gene expression and  
315 methylation levels adjusted for age, sex, cohort, and blood cell type composition. A second  
316 model was run without adjusting for blood cellular composition and it is only reported on the  
317 online web catalog, but not discussed in this manuscript. Although some of the unique  
318 associations of the unadjusted model might be real, others might be confounded by the large  
319 methylation and expression changes among blood cell types.

320 To ensure that CpGs paired to a higher number of Genes do not have higher chances of  
321 being part of an eQTM, multiple-testing was controlled at the CpG level, following a  
322 procedure previously applied in the Genotype-Tissue Expression (GTEx) project (Gamazon

323 et al., 2018). Briefly, our statistic used to test the hypothesis that a pair CpG-Gene is  
324 significantly associated is based on considering the lowest p-value observed for a given CpG  
325 and all its paired Gene (e.g., those in the 1 Mb window centered at the TSS). As we do not  
326 know the distribution of this statistic under the null, we used a permutation test. We  
327 generated 100 permuted gene expression datasets and ran our previous linear regression  
328 models obtaining 100 permuted p-values for each CpG-Gene pair. Then, for each CpG, we  
329 selected among all CpG-Gene pairs the minimum p-value in each permutation and fitted a  
330 beta distribution that is the distribution we obtain when dealing with extreme values (e.g.  
331 minimum) (Dudbridge and Gusnanto, 2008). Next, for each CpG, we took the minimum p-  
332 value observed in the real data and used the beta distribution to compute the probability of  
333 observing a lower p-value. We defined this probability as the empirical p-value of the CpG.  
334 Then, we considered as significant those CpGs with empirical p-values to be significant at  
335 5% false discovery rate using Benjamini-Hochberg method. Finally, we applied a last step to  
336 identify all significant CpG-Gene pairs for all eCpGs. To do so, we defined a genome-wide  
337 empirical p-value threshold as the empirical p-value of the eCpG closest to the 5% false  
338 discovery rate threshold. We used this empirical p-value to calculate a nominal p-value  
339 threshold for each eCpG, based on the beta distribution obtained from the minimum  
340 permuted p-values. This nominal p-value threshold was defined as the value for which the  
341 inverse cumulative distribution of the beta distribution was equal to the empirical p-value.  
342 Then, for each eCpG, we considered as significant all eCpG-Gene variants with a p-value  
343 smaller than nominal p-value.

## 344 Characterization of the child blood autosomal cis eQTM 345 catalogue

346 Wilcoxon tests were run to compare continuous variables (e.g., methylation range, CpG  
347 probe reliability, etc.) vs. categorical variables (e.g., low, medium, and high categories of  
348 methylation levels, eCpGs vs non eCpGs, etc.). We run a linear model to test the association

349 between the effect size and the distance between the CpG and the Gene's TSS. For this  
350 test, we compared the absolute value of the effect size vs  $\log_{10}$  of absolute value of the  
351 distance,

352 *Enrichment of eCpGs for regulatory elements:* Enrichment of eQTMs for regulatory elements  
353 were tested using Chi-square tests with non eQTMs as reference, unless otherwise stated.  
354 Results with a p-value  $<0.05$  were considered statistically significant. Annotation of eQTMs  
355 to regulatory elements (gene relative positions, CpG island relative positions, and blood  
356 ROADMAP chromatin states) is described in the section "DNA methylation assessment".  
357 Enrichment for CpGs classified in 3 groups based on their median methylation levels (low:  
358 0.0-0.3; medium:  $>0.3-0.7$ ; and high:  $>0.7-1.0$ ) was tested similarly.

359 *Enrichment of eCpGs for CpGs associated with phenotypic traits and exposures:* We also  
360 explored the enrichment of eQTMs for phenotypic traits and/or environmental exposures  
361 reported in the EWAS catalog (Battram et al., 2021) and the EWAS Atlas (Li et al., 2019).  
362 We used version 03-07-2019 of the EWAS catalog and selected those studies conducted in  
363 whole or peripheral blood of European ancestry individuals. We downloaded EWAS Atlas  
364 data on 27-11-2019 and selected those studies performed in whole blood or peripheral blood  
365 of European ancestry individuals or with unreported ancestry. Enrichment was tested as  
366 indicated above.

367 *Enrichment of eCpGs for age-variable CpGs:* We used results from the MeDALL and the  
368 Epidelta projects to test whether eQTMs were enriched for CpGs variable from birth to  
369 childhood and adolescence. For MeDALL we downloaded data from supplementary material  
370 of the following manuscript that assesses changes from 0 to 4y and from 4y to 8y (Xu et al.,  
371 2017). For Epidelta, we downloaded the full catalogue (version 2020-07-17) from their  
372 website (<http://epidelta.mrcieu.ac.uk/>). In Epidelta, we considered a CpG as age-variable if  
373 its p-value from model 1 that assesses linear changes from 0 to 17 years (variable  
374 M1.change.p) was  $<1e-7$  (Bonferroni threshold as suggested in the study). Variable CpGs



375 were classified as increased methylation if their change estimate (variable  
376 M1.change.estimate) was >0, and as decreased methylation, otherwise. Enrichment was  
377 tested as indicated above.

378 *Enrichment of eGenes for Gene Ontology - Biological Processes (GO-BP)*: We also tested  
379 whether eGenes were enriched for specific GO-BP terms using the *topGO* R package  
380 (RRID:SCR\_014798) (J, 2010) and using the genes annotated by Affymetrix in our dataset  
381 as background (58,254 Genes annotated to 23,054 Gene Symbols). We applied the  
382 *weight01* algorithm, which considers GO-BP terms hierarchy for p-values computation. GO-  
383 BP terms with q-value <0.001 were considered statistically significant.

## 384 Comparison of genes associated with eQTMs versus 385 annotation of eQTMs to the closest gene

386 We evaluated whether genes associated with eQTMs could be captured through the Illumina  
387 annotation, which links CpGs to the closest gene in a maximum distance of 1500 bp. For  
388 this, CpGs were annotated to Gene Symbols using the  
389 *IlluminaHumanMethylation450kanno.ilmn-12.hg19* R package (Hansen, n.d.), while Genes  
390 were annotated to Gene Symbols using the HTA-2.0 Transcript Cluster Annotations Release  
391 na36 annotation file from Affymetrix. Given that CpGs and Genes could be annotated to  
392 several genes, we considered that a CpG-Gene pair was annotated to the same gene if at  
393 least one of the genes annotated to the CpG was present among the genes in the HTA-2.0  
394 array. In total, we identified 327,931 CpG-Gene pairs annotated to the same gene, and thus  
395 that could be compared. Then, a Chi-square test was applied to compute whether eQTMs  
396 were enriched for these 327,931 comparable CpG-Gene pairs, using as background all 13M  
397 CpG-Gene pairs.

398 Next, we evaluated whether the relative position of the CpG in the genic region was related  
399 to the expression of the paired Gene. To do so, the comparable 327,931 CpG-Gene pairs

400 were expanded to 383,672 entries. Each entry represented a CpG-Gene pair annotated to a  
401 unique gene relative position. Thus, for instance, a CpG-Gene pair with the CpG annotated  
402 to two relative gene positions of the same gene was included as two entries, each time  
403 annotated to a different gene relative position. In this expanded CpG-Gene pair set, Chi-  
404 square tests were run to test the enrichment of eQTMs for gene relative positions, using the  
405 383,672 entries as background.

## 406 Evaluation of the genetic contribution on child blood autosomal 407 cis eQTMs

408 We used two approaches to evaluate the influence of genetic effects in child blood  
409 autosomal cis eQTMs. First, we analyzed heritability estimates of CpGs computed by Van  
410 Dongen and colleagues (van Dongen et al., 2016). Total additive and SNP-heritabilities were  
411 compared between eCpGs and non eCpGs, using a Wilcoxon test. We also run linear  
412 regressions between heritability measures (outcome) and eCpGs classified according to the  
413 number of eGenes they were associated with.

414 Second, we tested whether eCpGs were more likely regulated by SNPs than non eCpGs  
415 (i.e., whether they were enriched for meQTL). In order to define meQTLs in HELIX, we  
416 selected 9.9 M cis and trans meQTLs with a p-value  $<1e-7$  in the ARIES dataset consisting  
417 of data from children of 7 years old (Gaunt et al., 2016). Then, we tested whether this subset  
418 of 9.9 M SNPs were also meQTLs in HELIX by running meQTL analyses using *MatrixEQTL*  
419 R package (Shabalina, 2012), adjusting for cohort, sex, age, blood cellular composition and  
420 the first 20 principal components (PCs) calculated from genome-wide genetic data of the  
421 GWAS variability. We confirmed 2.8 M meQTLs in HELIX (p-value  $<1e-7$ ). Trans meQTLs  
422 represented  $<10\%$  of the 2.8 M meQTLs. Enrichment of eCpGs for meQTLs was computed  
423 using a Chi-square test, using non eCpGs as background.

424 Finally, we tested whether meQTLs were also eQTLs for the eGenes linked to the eCpGs.  
425 To this end, we run eQTL analyses (gene expression being the outcome and 2.8 M SNPs  
426 the predictors) with *MatrixEQTL* adjusting for cohort, sex, age, blood cellular composition  
427 and the first 20 GWAS PCs in HELIX. We considered as significant eQTLs the SNP-Gene  
428 pairs with p-value  $<1e-7$  and with the direction of the effect consistent with the direction of  
429 the meQTL and the eQTM.

## 430 Comparison with adult blood eQTM catalogues: GTP and 431 MESA

432 We compared our list of child blood autosomal cis eQTMs obtained in HELIX with the cis  
433 and trans eQTMs described in blood of two adult cohorts: GTP and MESA (Kennedy et al.,  
434 2018). DNA methylation was assessed with the Infinium HumanMethylation450K BeadChip  
435 (Illumina) in the 3 cohorts. In HELIX, gene expression was assessed with the Human  
436 Transcriptome Array 2.0 ST arrays (HTA 2.0) (Affymetrix), and in GTP and MESA with the  
437 HumanHT-12 v3.0 and v4.0 Expression BeadChip (Illumina).

438 For the comparison of eQTMs between adults and children, eGenes in the two studies were  
439 annotated to a common gene nomenclature, by using the Gene Symbol annotation provided  
440 by the authors from GTP and MESA, and the Gene Symbol provided by the Affymetrix  
441 annotation in HELIX. Some eQTMs involved Genes (HELIX) or gene probes (GTP and  
442 MESA) annotated to more than one gene (Gene Symbol); and also different Genes (HELIX)  
443 or gene probes (GTP and MESA) were annotated to the same Gene Symbol. To handle this  
444 issue, we split our comparison in two analyses.

445 First, we checked whether CpG-gene pairs reported in GTP and MESA were eQTMs  
446 (significant CpG-gene pairs) in HELIX. By doing this, the comparison was restricted to cis  
447 effects (as HELIX only considered cis effects). When a CpG-gene pair in GTP or MESA  
448 mapped to multiple CpG-gene pairs in HELIX, we only considered the CpG-gene pair with

449 the smallest p-value in HELIX. Next, Pearson's correlations between the effect sizes of the  
450 different studies were computed.

451 Second, we explored whether HELIX eQTM were also present in GTP and/or MESA. When  
452 a CpG-gene pair in HELIX mapped to multiple CpG-gene pairs in GTP and/or MESA, we  
453 only considered the CpG-gene pair with the smallest p-value in these cohorts. As a result,  
454 HELIX eQTMs were classified in age-shared (if present in adults at p-value  $<1e-05$ , in GTP  
455 and/or MESA) and children-specific (absent in adult cohorts). For these two subsets of  
456 eQTMs, enrichment for ROADMAP chromatin states, methylation measurement error, and  
457 distance from the eCpG to the eGene's TSS, was tested as explained above.

## 458 Data and software availability

459 The raw data used to generate the eQTM catalogue are not publicly available due to privacy  
460 restrictions but are available from the corresponding author on request. Catalogue of eQTMs  
461 described in this manuscript is publicly available at <https://helixomics.isglobal.org/>. Scripts to  
462 reproduce the analysis can be found in a public GitHub repository  
463 (<https://github.com/yocra3/methExprsHELIX/>) and as a supplementary file.

## 464 Results

### 465 Study population and molecular data

466 The study includes 823 children of European ancestry from the HELIX project with available  
467 blood DNA methylation and gene expression data. These children, enrolled in 6 cohorts,  
468 were aged between 6 and 11 years and the number of males and females was balanced  
469 (Table 1).

470 After quality control, our dataset consists of 386,518 CpGs and 58,254 transcript clusters  
471 (TCs) in autosomal chromosomes (from 1 to 22). TCs are defined as groups of one or more  
472 probes covering a region of the genome, reflecting all the exonic transcription evidence  
473 known for the region, and corresponding to a known or putative gene. Thus, we will refer  
474 TCs to Genes indistinctively. According to Affymetrix annotation, 23,054 of the Genes  
475 encoded a protein. To detect cis effects, we paired each Gene to all CpGs closer than 0.5  
476 Mb from its transcription start site (TSS), either upstream or downstream (1 Mb window  
477 centered at the TSS). In total, we obtained 13.6 M CpG-Gene pairs, where each CpG was  
478 paired to a median of 30 Genes; and each Gene was paired to a median of 162 CpGs  
479 (Figure 1 – figure supplement 1).

## 480 **Identification of autosomal cis eQTMs in children's blood**

481 We tested the association between DNA methylation and gene expression levels in the  
482 13.6 M autosomal CpG-Gene pairs through linear regressions adjusting for sex, age, cohort,  
483 and cellular composition. After correcting for multiple testing (see Material and Methods), we  
484 identified 39,749 statistically significant autosomal cis eQTMs in children's blood (0.29% of  
485 total CpG-Gene pairs). These eQTMs comprised 21,966 unique CpGs (5.7% of total CpGs)  
486 and 8,886 unique Genes (15.3% of total Genes), of which 6,288 were annotated as coding  
487 genes. For simplicity, we will refer to them as eQTMs (statistically significant associations of  
488 CpG-Gene pairs), eCpGs (CpGs involved in eQTMs), and eGenes (Genes involved in  
489 eQTMs). 23,355 eQTMs (58.8% of total) showed inverse associations, meaning that higher  
490 DNA methylation was associated with lower gene expression. In eQTMs, each eGene was  
491 associated with a median of 2 eCpGs, while each eCpG was associated with a median of 1  
492 eGene (Figure 1 – figure supplement 2). eCpGs presented higher methylation variability in  
493 the population (Figure 1 – figure supplement 3), and were measured with lower technical  
494 error (Sugden et al., 2020) (Figure 1 – figure supplement 4). Indeed, 13,278 eCpGs (60.4%  
495 of total) were measured with probes which had an intraclass correlation coefficient (ICC)

496 >0.4, which is indicative of reliable measurements. Moreover, eGenes had higher call rates  
497 (Figure 1 – figure supplement 5).

498 The complete catalogue of eQTMs can be downloaded from <https://helixomics.isglobal.org/>.

## 499 **Overview of autosomal cis eQTMs in children's blood**

### 500 *Distance from the eCpG to the eGene's TSS and effect size*

501 eCpGs tended to be close to the TSS of the targeted eGenes, being this distance <250Kb  
502 for 87.9% of all eQTMs (Figure 2A). Globally, the median distance between an eCpG and  
503 the TSS of its associated eGene was 1.1 kb (IQR = -33 kb; 65 kb), being eCpGs closer to  
504 the TSS in inverse eQTMs than in positive. The observed downstream shift could be  
505 explained because we chose the most upstream TSS for each Gene according to the  
506 Affymetrix HTAv2 annotation. A similar shift was observed for expression quantitative trait  
507 loci, eQTLs, (i.e., single nucleotide polymorphisms, SNPs, associated with gene expression)  
508 in the Genotype-Tissue Expression (GTEx) project (Gamazon et al., 2018).

509 We report the effect size of eQTMs as the  $\log_2$  fold change (FC) of gene expression per 0.1  
510 points increase in methylation (or 10 percentile increase). In absolute terms, the median  
511 effect size was 0.12, being the minimum 0.002 and the maximum 16.0, with 96.3% of the  
512 eQTMs with an effect size <0.5. A median effect size of 0.12 means that a change of 0.1  
513 points in methylation levels was associated with around a 9% increase/decrease of gene  
514 expression. We observed an inverse linear association between the eCpG-eGene's TSS  
515 distance and the effect size (p-value = 7.75e-9, Figure 2B); while we did not observe  
516 significant differences in effect size due to the relative orientation of the eCpG (upstream or  
517 downstream) with respect to the eGene's TSS (p-value = 0.68).

## 518 *Classification of eCpGs*

519 As shown in Table 2, we classified eCpGs into 5 types, by following 2 criteria: (1) the number  
520 of eGenes affected, distinguishing between mono eCpGs (associated with a unique eGene),  
521 and multi eCpGs (associated with  $\geq 2$  eGenes); and (2) the direction of the effect,  
522 distinguishing between inverse, positive and bivalent eCpGs (with inverse effects on some  
523 eGenes and positive effects on others). Mono inverse eCpGs were the most abundant type  
524 (36.8%) (Table 2). CpGs not associated with the expression of any Gene were named as  
525 non eCpGs. We used these categories in the subsequent analyses.

## 526 **Comparison of eGenes with the closest annotated gene**

527 A standard approach to interpret EWAS findings is to assume that a CpG regulates the  
528 expression of proximal genes. These genes are usually identified through the Illumina 450K  
529 annotation (Hansen, n.d.), which annotates a CpG to a gene when the CpG maps into the  
530 gene body, untranslated, or promoter region defined as  $<1,500$  bp upstream the TSS. We  
531 evaluated to which extent the Illumina 450K annotation captured the eQTMs identified in our  
532 catalogue.

533 First, we observed that CpG-Gene pairs where CpG and Gene were annotated to the same  
534 Gene Symbol were more likely eQTMs than CpG-Gene pairs annotated to different Gene  
535 Symbols or without gene annotation (OR = 11.90, p-value  $<2e-16$ ). Next, we assessed  
536 whether the gene annotated to the eCpG with the Illumina 450K annotation was coincident  
537 with the eGene found in our analysis. To answer this, we selected 14,797 eCpGs (67.4% of  
538 total eCpGs) annotated to Gene Symbols also present in the Affymetrix array, and thus  
539 comparable. In 7,808 out of these 14,797 eCpGs, the eCpG was associated with the  
540 expression of an eGene coincident with at least one of the Gene Symbols in Illumina's  
541 annotation (52.8% of eCpGs with comparable gene annotation, 35.5% of all eCpGs).

542 Finally, we explored whether the relative gene position of a CpG determines its association  
543 with gene expression. We selected the 327,931 CpG-Gene pairs with the CpG and Gene  
544 annotated to the same Gene Symbol. Within this subset, eCpGs were enriched for CpGs in  
545 5'UTRs and gene body positions, while depleted for CpGs in proximal promoters and  
546 3'UTRs (Figure 2 – figure supplement 1). Interestingly, we observed that inverse and  
547 positive eCpGs were enriched for CpGs located in different gene regions: inverse for CpGs  
548 in distal promoters (TSS1500) and 5'UTRs; positive for CpGs in gene bodies.

549 Overall, only around half of the eGenes targeted by the eQTMs could be identified by the  
550 Illumina 450K annotation. We also found that while eCpGs were enriched for TSS1500,  
551 5'UTRs, and gene body positions.

## 552 **Functional characterization of autosomal cis eQTMs in** 553 **children's blood**

### 554 *Enrichment of eCpGs for genomic regulatory elements*

555 We characterized eCpGs by evaluating their enrichment for diverse regulatory elements,  
556 including CpG island relative positions and 15 chromatin states retrieved from 27 blood cell  
557 types from the ROADMAP Epigenomics project (Roadmap Epigenomics Consortium et al.,  
558 2015). First, we found that eCpGs were depleted for CpG islands, while mostly enriched for  
559 CpG island shores, but also for shelves and open sea (Figure 3A). We did not observe  
560 relevant differences between inverse and positive eCpGs.

561 Second, we assessed whether eCpGs were enriched for ROADMAP blood chromatin states  
562 (Roadmap Epigenomics Consortium et al., 2015) (Figure 3B). eCpGs were enriched for  
563 several active states, such as enhancers or active transcription regions. Nonetheless, we  
564 observed some discrepancies between eCpGs subtypes: only inverse eCpGs were enriched  
565 for proximal promoter states while only positive eCpGs were depleted for transcription at 5'



566 and 3' (TxFlnk). In inactive chromatin states, both positive and inverse eCpGs were enriched  
567 for bivalent regulatory states (BivReg), while only positive eCpGs were enriched for  
568 repressed and weak repressed Polycomb regions (ReprPC, ReprPCWk) and quiescent  
569 regions (Quies).

570 Third, we also analyzed whether eCpGs had different methylation levels. We found that  
571 eCpGs were enriched for CpGs with medium (>0.3-0.7) methylation levels and depleted for  
572 CpGs with low (0-0.3) or high (>0.7-1) methylation levels (Figure 3C).

573 Finally, we wondered whether these enrichments could be affected by the bias introduced by  
574 methylation measurement error; thus, we repeated all the enrichment analyses only  
575 considering 75,836 CpGs measured with reliable probes (ICC >0.4) (Sugden et al., 2020)  
576 (Figure 3 – figure supplement 1). After this filtering, the enrichments for CpG island relative  
577 positions and for categories of CpGs according to their methylation levels changed  
578 substantially: eCpGs passed from being depleted to being enriched for CpG island positions  
579 (Figure 3 – figure supplement 1A), and from being enriched for CpGs with medium  
580 methylation levels to being enriched for CpGs with low methylation levels (Figure 3 – figure  
581 supplement 1C). On the contrary, the magnitudes of enrichments for most of the active  
582 chromatin states were increased (Figure 3 – figure supplement 1B); while enrichments of  
583 positive eCpGs for inactive states (ReprPoly and Quies) were reverted. Overall, selecting  
584 reliable CpG probes reduced the differences between inverse and positive eCpGs and  
585 resulted in enrichments for active chromatin states and depletions for inactive states.

### 586 *Gene-set enrichment analysis*

587 To identify which biological functions were regulated by our list of eQTMs, we ran gene-set  
588 enrichment analyses using the list of eGenes. 5,503 out of the 8,886 unique Gene Symbols  
589 annotated to eGenes were present in Gene Ontology - Biological Processes (GO-BP),  
590 leading to 52 enriched terms (q-value <0.001) (Table S1). As expected from the tissue  
591 analyzed, 50% of the terms were related to immune responses (N = 26), followed by terms

592 associated with cellular (N = 16) and metabolic (N = 10) processes. Among immune terms, 9  
593 of them were part of innate immunity, 9 of adaptive response, and 8 were related to  
594 general/other immune pathways. Most enriched GO-BP terms were also found when running  
595 the enrichment with the list of eGenes derived from eQTMs measured with reliable CpG  
596 probes (ICC >0.4) (Table S1).

### 597 *Enrichment for CpGs reported in the EWAS catalogues*

598 We assessed whether eCpGs were enriched for CpGs previously related to phenotypic traits  
599 and/or environmental exposures. To this end, we retrieved CpGs from EWAS performed in  
600 blood of European ancestry subjects: 143,384 CpGs from the EWAS catalog (Batram et al.,  
601 2021), and 54,599 CpGs from the EWAS Atlas (Li et al., 2019). We found that eCpGs were  
602 enriched for CpGs in these EWAS databases in comparison to non eCpGs. Although we  
603 observed larger odds ratios (ORs) for CpGs listed in the EWAS Atlas than for CpGs in the  
604 EWAS Catalog (Figure 3 – figure supplement 2A), this difference disappeared after  
605 removing CpGs with less reliable measurements (ICC <0.4) (Figure 3 – figure supplement  
606 2B).

607

## 608 **Genetic contribution to autosomal cis eQTMs in children's** 609 **blood**

### 610 *Additive and SNP heritability of eQTMs*

611 We hypothesized that genetic variation might regulate DNA methylation and gene  
612 expression in some of the autosomal cis eQTMs in children's blood. To test this, we used  
613 two measures of genetic influence: (1) heritability of blood DNA methylation levels for each  
614 CpG, calculated from twin designs (total additive heritability) and from genetic relationship

615 matrices (SNP heritability), as reported by Van Dongen and colleagues (van Dongen et al.,  
616 2016); and (2) methylation quantitative trait loci (meQTLs, SNPs associated with DNA  
617 methylation levels) identified in the ARIES dataset (Gaunt et al., 2016).

618 First, we found that eCpGs had higher total additive and SNP heritabilities than non eCpGs  
619 (median difference of 0.31 and 0.11, respectively, p-value  $<2e-16$  for both). Moreover, total  
620 additive and SNP heritabilities were higher for eCpGs associated with a larger number of  
621 eGenes (increase of 0.025 and 0.026 points per eGene, respectively, with a p-value  $<2e-16$   
622 for both) (Figure 4A and 4B). After removing CpG probes with unreliable measurements  
623 (ICC  $<0.4$ ), differences in median total additive heritability between eCpGs and non eCpGs  
624 were still present, but smaller (0.15, p-value  $<2e-16$ ); whereas differences in SNP  
625 heritabilities were maintained (0.11, p-value  $<2e-16$ ) (Figure 4 – figure supplement 1).

### 626 *Overlap with methylation and expression quantitative trait loci (meQTLs* 627 *and eQTLs)*

628 Second, we studied whether eCpGs were enriched for meQTLs, either in cis or trans. We  
629 analyzed 1,078,466 meQTLs identified in blood samples of 7-year-old children in the ARIES  
630 dataset and replicated in HELIX (see Material and Methods). These meQTLs affected the  
631 methylation of 36,671 CpGs through a total of 2,820,145 SNP-CpG pairs. 10,187 eCpGs  
632 (27.8% total eCpGs) presented at least 1 meQTL, being eCpGs enriched in CpGs  
633 associated with genetic variants (OR: 11.06, p-value  $<2e-6$ ). In addition, among CpGs with  
634 meQTLs, eCpGs were associated with a higher number of meQTLs (median: 74, IQR: 27;  
635 162) than non eCpGs (median: 32, IQR = 10; 77). Finally, eCpGs associated with a higher  
636 number of eGenes are more likely to be associated with at least one meQTL (Figure 4C).  
637 After removing CpG probes with unreliable measurements (ICC  $<0.4$ ), we observed the  
638 same trends, although the enrichment of eCpGs for CpGs with at least one meQTL was  
639 reduced (OR = 3.5, p-value  $<2.2e-16$ ) (Figure 4 – figure supplement 2). Finally, we observed

640 that eCpGs with at least one meQTL were measured with higher reliability (higher ICC) than  
641 eCpGs without any meQTL (Figure 4 – figure supplement 3).

642 We, then, determined whether meQTLs were also eQTLs for the eGenes. After multiple-  
643 testing correction, we identified 1,368,613 SNP-CpG-Gene trios with consistent direction of  
644 effect, and 12,799 with inconsistent direction. These formers comprised 16,055 unique  
645 eQTLs (40.4% of significant eQTLs); 8,503 unique eCpGs (38.7% of total eCpGs); and  
646 4,098 unique eGenes (46.1% of total eGenes), of which 3,154 were coding (50.2% of total  
647 coding eGenes). In these trios, eGenes were associated with a median of 2 eCpGs (IQR =  
648 1; 5) and 67 SNPs (IQR = 21; 149); whereas eCpGs were associated with a median of 1  
649 eGene (IQR = 1; 2) and 53 SNPs (IQR = 17; 124). One example of such a SNP-CpG-Gene  
650 trio is formed by rs11585123-cg15580684-TC01000080.hg.1 (*AJAP1*), in chromosome 10  
651 (Figure 4 – figure supplement 4).

652 Next, we run gene-set enrichment analyses with the 2,746 eGenes involved in these trios.  
653 We identified 35 significant GO-BP terms (q-value <0.001). Of these, 14 were related to  
654 immunity (6 innate, 4 adaptive immunity, and 4 general/other); 11 to cellular processes; and  
655 10 to metabolic processes (Table S1). In comparison to all eGenes, eGenes under genetic  
656 control had a reduction in the number of GO-BP terms involving immune and cellular  
657 functions (Table S2).

658 Overall, we found that a substantial part of the eQTLs seems to be under genetic control,  
659 and the SNPs associated with DNA methylation levels were also associated with gene  
660 expression levels.

661

## 662 **Influence of age on autosomal cis eQTM in children's**

### 663 **blood**

#### 664 *Enrichment for age-variable eQTMs*

665 To understand the association between changes in methylation and gene expression  
666 throughout life, first we evaluated whether eCpGs were enriched for CpGs with variable  
667 blood methylation levels from birth to childhood/adolescence. To this end, we retrieved the  
668 CpGs that vary with age from two databases: 14,150 CpGs with variable methylation levels  
669 in children between 0 and 8 years (9,647 with increased and 4,503 with decreased  
670 methylation) from the MeDALL project (Xu et al., 2017); and 244,283 CpGs with variable  
671 methylation levels in children and adolescents between 0 and 17 years (168,314 with  
672 increased and 75,969 with decreased methylation) from the Epidelta project (RH et al.,  
673 2021). Of note, 90% of the CpGs identified in the MeDALL project were also reported in the  
674 Epidelta. We found that eCpGs were enriched for age-variable CpGs in both MeDALL and  
675 Epidelta databases, but more markedly for CpGs reported in MeDALL (Figure 5A). In both  
676 databases, positive and inverse eCpGs showed stronger ORs for CpGs with increased and  
677 decreased methylation levels over age, respectively. After excluding CpG probes with  
678 unreliable measurements (ICC <0.4), MeDALL enrichments were reduced to the magnitude  
679 of Epidelta enrichments, while the differences between positive and inverse eCpGs were  
680 more evident (Figure 5 – figure supplement 1).

#### 681 *Overlap with autosomal eQTMs in adult blood*

682 We evaluated whether autosomal cis eQTMs in children's blood were consistent in adult  
683 populations. For this, we used data from the study of autosomal cis and trans eQTMs in  
684 adults' blood based on two cohorts: (1) GTP, whole blood and 333 samples; and (2) MESA,  
685 monocytes and 1,202 samples, by Kennedy and colleagues (Kennedy et al., 2018). The  
686 catalogue contains the summary statistics of all autosomal cis (<50 kb from the TSS) and

687 trans (otherwise) CpG-gene pairs at p-value  $<1e-5$ , although only CpG-gene associations at  
688 p-value  $<1e-11$  were considered significant eQTMs in their study. To compare their findings  
689 with ours, we mapped Genes and gene probes to Gene Symbols and compared CpG-gene  
690 pairs (see Material and Methods, Table S3).

691 We observed that 57.9% and 35.3% of eQTMs with p-value  $<1e-5$  in GTP and MESA were  
692 also eQTMs in HELIX, thus age-shared eQTMs (Figure 6B). More than 90% of age-shared  
693 eQTMs have the same direction in GTP/MESA than in HELIX (Table S4). In addition, effect  
694 sizes in GTP/MESA were correlated with effects sizes in HELIX (Table S4).

695 Only 5,471 (13.8%) of the eQTMs identified in HELIX children were reported in adult GTP or  
696 MESA catalogues at p-value  $<1e-5$  (Figure 6C). We explored whether eQTMs identified both  
697 in HELIX children and in adults (age-shared eQTMs) had different characteristics compared  
698 to eQTMs only found in children (child-specific eQTMs). Age-shared eQTMs involved 4,364  
699 eCpGs and 1,689 eGenes, whereas children-specific eQTMs involved 19,584 eCpGs and  
700 8,429 eGenes. Age-shared eCpGs had higher reliability (higher ICC) (Figure 5 – figure  
701 supplement 2) and tended to be closer to the TSS than child-specific eCpGs (Figure 5 –  
702 figure supplement 3). The enrichment for ROADMAP blood chromatin states (Roadmap  
703 Epigenomics Consortium et al., 2015) of age-shared and child-specific eCpGs in comparison  
704 to non eCpGs was quite similar (Figure 5 – figure supplement 4). Nonetheless, age-shared  
705 eCpGs showed higher ORs of enrichment for proximal promoters. Both types of eCpGs were  
706 enriched for meQTLs compared to non eCpGs, with the OR being stronger for age-shared  
707 eCpGs (OR = 20.7) than for child-specific eCpGs (OR = 10.3).

708 Overall, we found that eQTMs were enriched for CpGs whose methylation levels changed  
709 from birth to adolescence. The overlap between child and adult eQTMs was small: only  
710 13.8% of HELIX eQTMs had also been described in adults. Age-shared eCpGs tended to be  
711 proximal to the TSS, enriched for promoter chromatin states, and with stronger signals of  
712 genetic regulation.

## 713 **Discussion**

714 In this work, we present a blood autosomal cis eQTM catalogue in children. We identified  
715 39,749 eQTMs, representing 21,966 unique eCpGs and 8,886 unique eGenes (6,288 of  
716 which were coding). 23,355 eQTMs (58.8% of all eQTMs) showed inverse associations. A  
717 substantial fraction was influenced by genetic variation, and the overlap with eQTMs  
718 reported in adults was small.

719 The characteristics of the autosomal cis eQTMs in children's blood were highly consistent  
720 with patterns previously described in other studies. Most of the eCpGs tended to be proximal  
721 to the eGene's TSS (Kennedy et al., 2018; Leland Taylor et al., 2019). The magnitude of the  
722 effect seemed to be proportional to the distance between the eCpG and the eGene's TSS,  
723 but this association was weak. Although higher DNA methylation is assumed to lead to lower  
724 expression, we found that around 40% of eQTMs were positively associated with gene  
725 expression. This percentage is in line with previous results from different tissues (Gutierrez-  
726 Arcelus et al., 2015, 2013; Küpers et al., 2019). Inverse and positive eCpGs tended to be  
727 localized in enhancers and other active regulatory regions and not in CpG islands, a pattern  
728 that was also previously reported (Gutierrez-Arcelus et al., 2015; Küpers et al., 2019).  
729 Despite these common locations, inverse eCpGs were specifically found around active TSSs  
730 (including the distal promoter and the 5'UTR), while positive eCpGs were localized in gene  
731 body regions. These results highlight the importance of the genomic context to infer the  
732 direction of the association between DNA methylation and gene expression (Kennedy et al.,  
733 2018). We want to point out that the causal relationship between DNA methylation and gene  
734 expression cannot be definitely inferred from our study. Indeed, there is some evidence  
735 suggesting that DNA methylation could be a consequence of gene expression, as opposed  
736 to the often assumed concept that regulation of gene expression is mediated by DNA  
737 methylation (Gutierrez-Arcelus et al., 2013; Jones, 2012).

738 eQTMs can be influenced by genetic variation (Lu et al., 2019). In HELIX, eCpGs linked to  
739 the expression of several eGenes had higher heritabilities and were associated with a higher  
740 number of meQTLs than non eCpGs. This could suggest that eCpGs that regulate the  
741 expression of several genes, the so-called master regulators, are more prone to be  
742 themselves regulated by genetic variation. We, then, searched for SNPs simultaneously  
743 associated with DNA methylation (meQTLs) and gene expression (eQTLs) in our data. We  
744 identified 1.3 M SNP-CpG-Gene trios with consistent direction of the effect. Interestingly, the  
745 number of GO-BP terms related to immune and cellular functions was reduced for eGenes  
746 under genetic control, in comparison to all eGenes; on the contrary, the number of GO-BP  
747 terms involving metabolic processes was maintained. This may suggest that the influence of  
748 environmental factors is more relevant for immune pathways, while genetic factors might be  
749 more determinant in regulating metabolic processes in blood cells. Given the non-negligible  
750 effect of genetics in eQTMs, we would advise studying the effect of genetic variants on the  
751 association between environmental factors or phenotypic traits and DNA methylation.

752 In order to know how eQTMs behave along life-course, we compared blood autosomal cis  
753 eQTMs identified in HELIX children with cis and trans eQTMs reported by Kennedy and  
754 colleagues in whole blood and monocytes from adult populations (Kennedy et al., 2018). We  
755 found that only 13.8% of the autosomal eQTMs in children's blood were also reported in  
756 adults. Similarly, a modest proportion of adult blood eQTMs was present in children (58%  
757 from GTP and 35% from MESA). This small overlap between adult and child eQTMs has  
758 different explanations: methodological issues, such as gene expression platforms with low  
759 overlap; statistical methods and statistical power; cohort-specific environmental exposures;  
760 and cellular composition. Unsurprisingly, HELIX and MESA presented the highest  
761 divergence, as HELIX assessed eQTMs in whole blood and MESA in monocytes. Despite  
762 the effect of these methodological and confounding factors, it is known that DNA methylation  
763 and gene expression change with age (Melé et al., 2015; RH et al., 2021; Xu et al., 2017);  
764 consequently, we could expect only partial overlap between adult and child eQTMs. The



765 short list of age-shared eCpGs tended to encompass CpGs located in promoters and  
766 regulated by genetic variants. Moreover, the overall location of eQTMs in regulatory  
767 elements was similar between adults and children (Gutierrez-Arcelus et al., 2015; Küpers et  
768 al., 2019). This could represent a specific characteristic of eQTMs that are persistent over  
769 time. An alternative explanation is that this kind of eQTMs (genetically regulated and close to  
770 the TSS) are easier to be detected and shared among any two studies because they show  
771 stronger effects. Finally, we observed that HELIX eQTMs usually involved CpGs whose  
772 methylation varied between birth and childhood/adolescence, and they tended to activate  
773 rather than inactivate transcription over this period. Also, they were enriched for CpGs found  
774 to be related to environmental factors and phenotypic traits in the EWAS Atlas and EWAS  
775 Catalog.

776 As previously described (Sugden et al., 2020), CpG probes have different measurement  
777 error and thus different reliability and reproducibility. Consequently, CpGs measured with  
778 less error have more chances of being found associated with traits and thus reported in  
779 EWAS catalogues. In HELIX, we found that CpG probe ICC was higher for these different  
780 cases: for eCpGs, in comparison to non eCpGs; for age-shared eCpGs, in comparison to  
781 children-specific eCpGs; and for eCpGs with meQTLs, in comparison to eCpGs without  
782 meQTLs. In this line, enrichments of eCpGs for CpGs listed in the EWAS Atlas or in the  
783 MeDALL project were markedly attenuated when only considering CpGs measured with  
784 good reliability. Moreover, CpG probe reliability is dependent on DNA methylation level and  
785 variance (highly unmethylated or highly methylated CpGs, which tend to have low variances,  
786 are measured with more error); and genomic regulatory elements are characterized by  
787 particular methylation levels. Therefore, this biased the enrichments for regulatory elements.  
788 For instance, after considering only reliable probes, the distribution of eQTMs in CpG island  
789 relative positions changed completely (Figure 3 – figure supplement 1). Moreover, the  
790 enrichments for active chromatin states were amplified and differences between inverse and  
791 positive eCpGs attenuated.

792 Our study of autosomal cis eQTM in children's blood has several strengths compared to  
793 previous eQTM studies. First, we report all CpG-gene pairs we tested in our analysis, as  
794 opposed to existing blood eQTM catalogues which only reported pairs passing a given p-  
795 value threshold (Bonder et al., 2017; Kennedy et al., 2018). Reporting all pairs tested allows  
796 replication and meta-analyses, reducing publication bias. Second, we report which eQTMs  
797 are influenced by genetic variation, and researchers can take this into account when  
798 exploring the relationship between methylation and expression in their data. Finally, as  
799 others (Wu et al., 2018), we describe that only around half of the CpG-Gene relationships  
800 are captured through annotation to the closest gene. Therefore, our eQTM catalogue  
801 becomes an essential and powerful tool to help researchers interpret their EWAS, with a  
802 particular focus on childhood.

803 The catalogue also has some limitations. First, it only covers a fraction of all CpG-Gene  
804 pairs, as both the methylation and gene expression arrays have limited resolution.  
805 Nonetheless, the catalogue will be useful for most researchers as the methylation array is  
806 widely used, and the gene expression array covers almost all the coding genes. Second, the  
807 catalogue does not include sex chromosomes which require more complex analyses to  
808 address X-inactivation and sex-specific effects that will be addressed in future studies. Third,  
809 due to statistical power limitations, only cis effects were tested. Despite that, we observed  
810 that eCpGs tended to be close to the gene they regulate, so the catalogue is expected to  
811 cover most of the CpG-Gene associations. Fourth, effect sizes should be considered with  
812 caution as the association between DNA methylation and gene expression might be non-  
813 linear, and the effect of outlier values was not systematically explored (Johnson et al., 2017).  
814 Fifth, models were adjusted for blood cell type composition and, while this has allowed us to  
815 control for major differences in methylation and gene expression among blood cell types, it  
816 might also have resulted in over-adjustment in some CpG-Gene pairs. Moreover, the  
817 analysis of bulk data might have limited the identification of eQTMs specific to a subset of  
818 blood cell types, the identification of which would need more sophisticated statistical and/or

819 experimental methods. Finally, we acknowledge that the catalogue will be useful for  
820 biological interpretation of EWAS if it is true that DNA methylation is not a mere mark of cell  
821 memory to past exposures (without transcriptional consequences or with time-limited ones)  
822 (Tsai et al., 2018).

823 In summary, besides characterizing child blood autosomal cis eQTMs and reporting how  
824 they are affected by genetics and age, we provide a unique public resource: a catalogue with  
825 13.6 M CpG-gene pairs and of 1.3 M SNP-CpG-gene trios (<https://helixomics.isglobal.org/>).  
826 This information will improve the biological interpretation of EWAS findings.

## 827 **Tables**

### 828 **Table 1. Descriptive of the study population.**

829 BiB: Born in Bradford study (UK). EDEN: Étude des Déterminants pré et postnatals du  
830 développement et de la santé de l'Enfant (France). KANC: Kaunus cohort (Lithuania). MoBa:  
831 Norwegian Mother, Father and Child Cohort Study (Norway). RHEA: Mother Child Cohort  
832 study (Greece). INMA: Infancia y Medio Ambiente cohort (Spain).

Variable	BiB	EDEN	KANC	MoBa	RHEA	INMA	All
N (%)	80 (9.7%)	80 (9.7%)	143 (17.4%)	188 (22.9%)	154 (18.7%)	178 (21.6%)	823 (100%)
Female (%)	36 (45%)	35 (43.8%)	64 (44.8%)	88 (46.8%)	69 (44.8%)	80 (44.9%)	372 (45.2%)
Male (%)	44 (55%)	45 (56.2%)	79 (55.2%)	100 (53.2%)	85 (55.2%)	98 (55.1%)	451 (54.8%)
Age, in years (IQR)	6.65 (6.44- 6.84)	10.76 (10.37- 11.22)	6.40 (6.12- 6.88)	8.53 (8.17- 8.83)	6.45 (6.36- 6.62)	8.84 (8.44- 9.21)	8.06 (6.49- 8.86)

Natural Killer cells (IQR)	0.01 (0.00-0.03)	0.02 (0.00-0.04)	0.04 (0.01-0.07)	0.02 (0.00-0.07)	0.01 (0.00-0.03)	0.03 (0.01-0.05)	0.02 (0.00-0.05)
B-cell (IQR)	0.12 (0.11-0.15)	0.09 (0.07-0.11)	0.11 (0.09-0.13)	0.11 (0.09-0.14)	0.14 (0.11-0.16)	0.10 (0.08-0.13)	0.11 (0.09-0.14)
CD4+ T-cell (IQR)	0.21 (0.18-0.25)	0.16 (0.14-0.20)	0.17 (0.14-0.21)	0.21 (0.17-0.25)	0.20 (0.16-0.26)	0.17 (0.14-0.21)	0.19 (0.15-0.23)
CD8+ T-cell (IQR)	0.13 (0.11-0.17)	0.11 (0.08-0.13)	0.13 (0.10-0.16)	0.14 (0.11-0.17)	0.14 (0.11-0.16)	0.12 (0.09-0.14)	0.13 (0.10-0.16)
Monocytes (IQR)	0.09 (0.07-0.10)	0.09 (0.07-0.11)	0.08 (0.06-0.09)	0.08 (0.07-0.10)	0.09 (0.07-0.10)	0.09 (0.07-0.11)	0.08 (0.07-0.10)
Granulocytes (IQR)	0.41 (0.35-0.47)	0.52 (0.47-0.56)	0.46 (0.40-0.53)	0.41 (0.32-0.48)	0.41 (0.34-0.48)	0.48 (0.42-0.55)	0.44 (0.37-0.52)

833 Continuous variables are expressed as mean and interquartile range (IQR).

834

835 **Table 2. Classification of eCpGs by type.**

836 Percentages refer to the total number of eCpGs.

	Inverse (N, %)	Positive (N, %)	Bivalent (N, %)	Total (N, %)
Mono	8,084 (36.8%)	5,681 (25.9%)	0, by definition	13,765 (62.7%)
Multi	3,738 (17.0%)	2,400 (10.9%)	2,063 (9.4%)	8,201 (37.3%)
Total	11,822 (53.8%)	8,081 (36.8%)	2,063 (9.4%)	21,966 (100%)

837

## 838 **Figure legends**

839 **Figure 1. Analysis workflow.** The figure summarizes the analyses conducted in this study. The first  
 840 step was (1) the identification of blood autosomal cis eQTM (1 Mb window centered at the  
 841 transcription start site, TSS, of the gene) in 823 European ancestry children from the HELIX project,  
 842 by a linear model adjusted for age, sex, cohort, and blood cell type proportions. All the associations  
 843 are reported in the web catalogue ([www.helixomics.isglobal.org](http://www.helixomics.isglobal.org)). Then, (2) we explored the distance  
 844 from the eCpG (CpG involved in an eQTM) to eGene's TSS (gene involved in an eQTM), the effect

845 size of the association, and classified eCpGs in different types. Next, (3) we evaluated the proportion  
846 of eGenes potentially inferred through annotation of eCpGs to the closest gene. Finally, (4) we  
847 functionally characterized eCpGs and eGenes; (5) assessed the contribution of genetic variants; and  
848 (6) evaluated the influence of age.

849 **Figure 1 – figure supplement 1. Distribution of Genes and CpGs in all CpG-Gene pairs.** A)  
850 Distribution of the number of Genes paired with each CpG. The y-axis represents the number of CpGs  
851 that are paired with a given number of Genes, indicated in the x-axis. The vertical line marks the  
852 median of the distribution. Each CpG was paired to a median of 30 Genes (IQR: 20; 46). B)  
853 Distribution of the number of CpGs paired with each Gene. The y-axis represents the number of  
854 Genes that are paired with a given number of CpGs, indicated in the x-axis. The vertical line marks  
855 the median of the distribution. Each Gene was paired to a median of 162 CpGs (IQR: 93; 297).

856 **Figure 1 – figure supplement 2. Distribution of eGenes and eCpGs in autosomal cis eQTMs.** A)  
857 Distribution of the number of eGenes paired with each eCpG in eQTMs. The y-axis represents the  
858 number of eCpGs that are paired with a given number of eGenes, indicated in the x-axis. Each eCpG  
859 was associated with a median of 1 eGene (IQR = 1; 2). B) Distribution of the number of eCpGs paired  
860 with each eGenes in eQTMs. The y-axis represents the number of eGenes that are paired with a  
861 given number of eCpGs, indicated in the x-axis. Each eGene was associated with a median of 2  
862 eCpGs (IQR = 1; 5).

863 **Figure 1 – figure supplement 3. DNA methylation range by CpG type.** CpGs were classified in:  
864 eCpGs (CpGs associated with gene expression, N=21,966, in grey) and non eCpGs (N=364,452, in  
865 white). Methylation range was computed as the difference between the methylation values in  
866 percentile 1 and percentile 99 (Lin et al., 2017).

867 **Figure 1 – figure supplement 4. Probe reliability by CpG type.** CpGs were classified in: eCpGs  
868 (CpGs associated with gene expression, N=21,966, in grey) and non eCpGs (N=364,452, in white).  
869 Probe reliability was based on intraclass correlation coefficients (ICC) obtained from (Sugden et al.,  
870 2020).

871 **Figure 1 – figure supplement 5. Genes call rate distribution by Gene type.** Genes were classified  
872 in: eGenes (Genes associated DNA methylation, N=8,886, in grey) and non eGenes (N=51,806, in  
873 white). For a given Gene, call rate is the proportion of children with gene expression levels over the  
874 background noise.

875 **Figure 2. Distance between CpG and Gene's TSS and effect size in child blood autosomal cis**  
876 **eQTMs.** A) Distribution of the distance between CpG and Gene's TSS by eQTM type. CpG-Gene  
877 pairs were classified in non eQTMs (black); inverse eQTMs (yellow); and positive eQTMs (green). The  
878 x-axis represents the distance between the CpG and the Gene's TSS (kb). Non eQTMs median  
879 distance: -0.013 kb (interquartile range - IQR = -237; 236). Positive eQTMs median distance: -4.9 kb  
880 (IQR = -38; 79). Inverse eQTMs median distance: -0.7 kb (IQR = -29; 54). B) Effect size versus eCpG-  
881 Gene's TSS distance in eQTMs. The x-axis represents the distance between the eCpG and the  
882 eGene's TSS (kb). The y-axis represents the effect size as the log<sub>2</sub> fold change in gene expression  
883 produced by a 0.1 increase in DNA methylation (or 10 percentile increase). To improve visualization,  
884 a 99% winsorization has been applied to log<sub>2</sub> fold change values: values more extreme than 99%  
885 percentile (in absolute value) have been changed for the 99% quantile value (in absolute value).  
886 eQTMs are classified in inverse (yellow) and positive (green). Each eQTM is represented by one dot.  
887 The darker the color, the more dots overlapping, and so the higher the number of eQTMs with the  
888 same effect size and eCpG-eGene's TSS distance.

889 **Figure 2 – figure supplement 1. Enrichment of eCpGs for gene relative positions.** We selected  
890 the subset of 327,931 CpG-Gene pairs where the CpG and the Gene were annotated to the same  
891 gene. Enrichment was computed for all eCpGs in this subset, and for inverse and positive eCpGs.

892 Genic regions are classified in distal promoter from 200 to 1,500 bp (TSS1500); proximal promoter up  
893 to 200 bp (TSS200), 5' untranslated region (5'UTR); 1st exon; gene body; and 3' untranslated region  
894 (3'UTR). The y-axis represents the odds ratio (OR) of the enrichment. For all gene relative positions,  
895 the enrichment was computed against CpG-Gene pairs with CpG and Gene annotated to the same  
896 gene that were not eQTMs.

897 **Figure 3. Enrichment of cis autosomal eCpGs in children's blood for different regulatory**  
898 **elements.** eCpGs were classified in all (grey), inverse (yellow), and positive (green). The y-axis  
899 represents the odds ratio (OR) of the enrichment. In all cases, the enrichment was computed against  
900 non eCpGs. A) Enrichment for CpG island relative positions: CpG island, N- and S-shore, N- and S-  
901 shelf, and open sea. B) Enrichment for ROADMAP blood chromatin states (Roadmap Epigenomics  
902 Consortium et al., 2015): active TSS (TssA); flanking active TSS (TssAFlnk); transcription at 5' and 3'  
903 (TxFlnk); transcription region (Tx); weak transcription region (TxWk); enhancer (Enh); genic enhancer  
904 (EnhG); zinc finger genes and repeats (ZNF.Rpts); flanking bivalent region (BivFlnx); bivalent  
905 enhancer (EnhBiv); bivalent TSS (TssBiv); heterochromatin (Het); repressed Polycomb (ReprPC);  
906 weak repressed Polycomb (ReprPCWk); and quiescent region (Quies). Chromatin states can be  
907 grouped in active transcription start site proximal promoter states (TssProxProm), active transcribed  
908 states (ActTrans), enhancers (Enhancers), bivalent regulatory states (BivReg), and repressed  
909 Polycomb states (ReprPoly). C) Enrichment for categories of CpGs with different median methylation  
910 levels: low (0-0.3), medium (0.3-0.7), and high (0.7-1) (Huse et al., 2015).

911 **Figure 3 – figure supplement 1. Enrichment of eCpGs with reliable measurement for different**  
912 **regulatory elements.** Only eCpGs with reliable measurements (ICC >0.4) were considered (Sugden  
913 et al., 2020). eCpGs were classified in all (grey), inverse (yellow), and positive (green). The y-axis  
914 represents the odds ratio (OR) of the enrichment. In all cases, the enrichment was computed against  
915 non eCpGs. A) Enrichment for CpG island relative positions: CpG island, N- and S-shore, N- and S-  
916 shelf, and open sea. B) Enrichment for ROADMAP blood chromatin states (Roadmap Epigenomics  
917 Consortium et al., 2015): active TSS (TssA), flanking active TSS (TssAFlnk), transcription at 5' and 3'  
918 (TxFlnk), transcription region (Tx), weak transcription region (TxWk), enhancer (Enh); genic enhancer  
919 (EnhG), zinc finger genes and repeats (ZNF.Rpts), flanking bivalent region (BivFlnx), bivalent  
920 enhancer (EnhBiv), bivalent TSS (TssBiv), heterochromatin (Het), repressed Polycomb (ReprPC),  
921 weak repressed Polycomb (ReprPCWk), and quiescent region (Quies). Chromatin states can be  
922 grouped in active transcription start site proximal promoter states (TssProxProm), active transcribed  
923 states (ActTrans), enhancers (Enhancers), bivalent regulatory states (BivReg) and repressed  
924 Polycomb states (ReprPoly). C) Enrichment for groups of CpGs with different median methylation  
925 levels: low (0-0.3), medium (0.3-0.7), and high (0.7-1) (Huse et al., 2015).

926 **Figure 3 – figure supplement 2. Enrichment of autosomal cis eCpGs in children's blood for**  
927 **CpGs reported to be associated with phenotypic traits and/or environmental exposures.**  
928 Enrichment for CpGs present in EWAS datasets: the EWAS Atlas (Li et al., 2019), and the EWAS  
929 Catalog (Battaram et al., 2021). eCpGs were classified in all (grey), inverse (yellow), and positive  
930 (green). In all cases, the enrichment was computed against non eCpGs. The y-axis represents the  
931 odds ratio (OR) of the enrichment. A) Enrichment considering all CpGs. B) Enrichment considering  
932 only CpGs measured with reliable probes (ICC >0.4) (Sugden et al., 2020). intraclass correlation  
933 coefficient.

934 **Figure 4. Genetic contribution to autosomal cis eQTMs in children's blood.** CpGs were grouped  
935 by the number of Genes they were associated with, where 0 means that a CpG was not associated  
936 with any Gene (non eCpG). A) Total additive heritability and B) SNP heritability as inferred by Van  
937 Dongen and colleagues (van Dongen et al., 2016). The y-axis represents heritability and the x-axis  
938 each group of CpGs associated with a given number of Genes. C) Proportion of CpGs having a  
939 meQTL (methylation quantitative trait locus), by each group of CpGs associated with a given number  
940 of Genes.

941 **Figure 4 – figure supplement 1. Heritability of methylation levels in CpGs with reliable**  
942 **measurements.** Only CpGs measured with reliable probes (ICC >0.4) were considered (Sugden et  
943 al., 2020). CpGs were grouped by the number of Genes they were associated with, where 0 means  
944 that a CpG was not associated with any Gene (non eCpGs, in white). A) Total additive heritability as  
945 inferred by Van Dongen and colleagues (van Dongen et al., 2016), by each group of CpGs associated  
946 with a given number of Genes. B) SNP heritability as inferred by Van Dongen and colleagues (van  
947 Dongen et al., 2016), by each group of CpGs associated with a given number of Genes.

948 **Figure 4 – figure supplement 2. Proportion of CpGs having a meQTL (methylation quantitative**  
949 **trait loci) among CpGs with reliable measurements.** Only CpGs measured with reliable probes  
950 (ICC >0.4) were considered (Sugden et al., 2020). CpGs were grouped by the number of Genes they  
951 were associated with.

952 **Figure 4 – figure supplement 3. Probe reliability in autosomal cis eCpGs according to**  
953 **association with genetic variants.** eCpGs were classified in two groups, depending on whether their  
954 methylation values were associated with any genetic variant. Probe reliabilities were based on  
955 intraclass correlations (ICCs) obtained from (Sugden et al., 2020).

956 **Figure 4 – figure supplement 4. Example of a trio of SNP-CpG-Gene.** A) Methylation levels  
957 (cg15580684) by SNP genotypes (rs11585123). B) Gene expression levels (TC01000080.hg.1,  
958 AJAP1 gene) by SNP genotypes (rs11585123). C) Correlation between gene expression  
959 (TC01000080.hg.1, AJAP1 gene) and methylation levels (cg15580684).

960 **Figure 5. Influence of age on autosomal cis eQTMs in children’s blood.** A) Enrichment of eCpGs  
961 for CpGs with age-variable methylation levels, in comparison to non eCpGs. eCpGs were classified in  
962 all (grey); inverse (yellow); and positive (green). Age-variable CpGs were retrieved from the MeDALL  
963 project (from birth to childhood (Xu et al., 2017)) and from the Epidelta project (from birth to  
964 adolescence (RH et al., 2021)). They were classified in variable (CpGs with methylation levels that  
965 change with age); decreased (CpGs with methylation levels that decrease with age); and increased  
966 (CpGs with methylation levels that increase with age). The y-axis represents the odds ratio (OR) of  
967 the enrichment. B) Overlap between autosomal cis/trans eQTMs identified in adults (GTP: whole  
968 blood; MESA: monocytes) (Kennedy et al., 2018) with cis eQTMs identified in children (HELIX: whole  
969 blood). All CpG-gene pairs reported at p-value <1e-5 in GTP or MESA that could be compared with  
970 pairs in HELIX are shown. C) Overlap between blood autosomal cis eQTMs identified in HELIX  
971 children with cis/trans eQTMs identified in adults (GTP: whole blood; MESA: monocytes) (Kennedy et  
972 al., 2018). All CpG-gene pairs in HELIX that could be compared with pairs in GTP or MESA are  
973 shown. Note: The comparison has been split into two plots because one eGene in HELIX can be  
974 mapped to different expression probes in GTP and MESA, and vice-versa. Only comparable CpG-  
975 Gene pairs are shown (see Material and Methods).

976 **Figure 5 – figure supplement 1. Enrichment of eCpGs with reliable measurements for CpGs**  
977 **with age-variable methylation levels.** Only CpGs with reliable measurements (ICC >0.4) were  
978 considered (Sugden et al., 2020). eCpGs were classified in all (grey), inverse (yellow); and positive  
979 (green). Age-variable CpGs were retrieved from the MeDALL project (from birth to childhood (Xu et  
980 al., 2017)) and the Epidelta project (from birth to adolescence (RH et al., 2021)), and they were  
981 classified in: variable (CpGs with methylation levels that change with age), decreased (CpGs with  
982 methylation levels that decrease with age), and increased (CpGs with methylation levels that increase  
983 with age). The y-axis represents the odds ratio (OR) of the enrichment. For all eCpG types, the  
984 enrichment was computed against non eCpGs.

985 **Figure 5 – figure supplement 2. Probe reliability in eCpGs according to overlap with adult**  
986 **eQTMs.** eCpGs were classified in age-shared eCpGs (eCpGs identified in HELIX children and also in  
987 adults from MESA and/or GTP studies, in red); and child-specific eCpGs (eCpGs only identified in

988 HELIX children and not in the adult cohorts, in blue). Probe reliabilities were based on intraclass  
989 correlation coefficients (ICCs) obtained from (Sugden et al., 2020).

990 **Figure 5 – figure supplement 3. Distribution of the distance between CpG-Gene’s TSS by eQTM**  
991 **type.** eQTMs were classified in age-shared (eQTMs identified in HELIX children and also in adults  
992 from MESA or GTP studies, in red); and child-specific (eQTMs only identified in HELIX children and  
993 not in adult cohorts, in blue). Distance between eCpG and eGene’s TSS is expressed in kb. Age-  
994 shared eQTMs median distance: 1.2 kb (IQR: -2.4; 35.4 kb). Child-specific eQTMs median distance: -  
995 1.1 kb (IQR: -39.4; 70.7 kb).

996 **Figure 5 – figure supplement 4. Enrichment of age-shared and child-specific eCpGs for blood**  
997 **ROADMAP blood chromatin states.** eCpGs were classified in age-shared (eCpGs identified in  
998 HELIX children and also in adults from MESA or GTP studies, in red); and child-specific (eCpGs only  
999 identified in HELIX children and not in adult cohorts, in blue). ROADMAP blood chromatin states  
1000 (Roadmap Epigenomics Consortium et al., 2015) are: active TSS (TssA), flanking active TSS  
1001 (TssAFlnk), transcription at 5' and 3' (TxFlnk), transcription region (Tx), weak transcription region  
1002 (TxWk), enhancer (Enh); genic enhancer (EnhG), zinc finger genes and repeats (ZNF.Rpts), flanking  
1003 bivalent region (BivFlnx), bivalent enhancer (EnhBiv), bivalent TSS (TssBiv), heterochromatin (Het),  
1004 repressed Polycomb (ReprPC), weak repressed Polycomb (ReprPCWk), and quiescent region  
1005 (Quies). Chromatin states can be grouped in active transcription start site proximal promoter states  
1006 (TssProxProm), active transcribed states (ActTrans), bivalent regulatory states (BivReg) and  
1007 repressed Polycomb states (ReprPoly). The y-axis represents the odds ratio (OR) of the enrichment.  
1008 For each regulatory element, the enrichment was computed against non eCpGs.

1009

## 1010 **File legends**

1011 **Supplementary tables (HELIX\_MethExpr\_SupTables.xlsx):** File with supplementary tables S1-S4.

1012 **Source code file (SupplementaryCode.zip):** compressed file with the code used to run the analyses  
1013 and generate the tables and figures.

## 1014 **Competing interests**

1015 The authors declare that they have no competing interests.

## 1016 **Acknowledgments**

1017 The authors acknowledge the contribution of all the HELIX children and their families.



## 1018 Funding

1019 The study has received funding from the European Community's Seventh Framework  
1020 Programme (FP7/2007-2006) under grant agreement no 308333 (HELIX project); the H2020-  
1021 EU.3.1.2. - Preventing Disease Programme under grant agreement no 874583 (ATHLETE  
1022 project); from the European Union's Horizon 2020 research and innovation programme  
1023 under grant agreement no 733206 (LIFECYCLE project), and from the European Joint  
1024 Programming Initiative "A Healthy Diet for a Healthy Life" (JPI HDHL and Instituto de Salud  
1025 Carlos III) under the grant agreement no AC18/00006 (NutriPROGRAM project). The  
1026 genotyping was supported by the project PI17/01225, funded by the Instituto de Salud  
1027 Carlos III and co-funded by European Union (ERDF, "A way to make Europe") and the  
1028 Centro Nacional de Genotipado-CEGEN (PRB2-ISCI).

1029 BiB received core infrastructure funding from the Wellcome Trust (WT101597MA) and a joint  
1030 grant from the UK Medical Research Council (MRC) and Economic and Social Science  
1031 Research Council (ESRC) (MR/N024397/1). INMA data collections were supported by  
1032 grants from the Instituto de Salud Carlos III, CIBERESP, and the Generalitat de Catalunya-  
1033 CIRIT. KANC was funded by the grant of the Lithuanian Agency for Science Innovation and  
1034 Technology (6-04-2014\_31V-66). The Norwegian Mother, Father and Child Cohort Study is  
1035 supported by the Norwegian Ministry of Health and Care Services and the Ministry of  
1036 Education and Research. The Rhea project was financially supported by European projects  
1037 (EU FP6-2003-Food-3-NewGeneris, EU FP6. STREP Hiwate, EU FP7 ENV.2007.1.2.2.2.  
1038 Project No 211250 Escape, EU FP7-2008-ENV-1.2.1.4 Envirogenomarkers, EU FP7-  
1039 HEALTH-2009- single stage CHICOS, EU FP7 ENV.2008.1.2.1.6. Proposal No 226285  
1040 ENRIECO, EU- FP7- HEALTH-2012 Proposal No 308333 HELIX), and the Greek Ministry of  
1041 Health (Program of Prevention of obesity and neurodevelopmental disorders in preschool  
1042 children, in Heraklion district, Crete, Greece: 2011-2014; "Rhea Plus": Primary Prevention  
1043 Program of Environmental Risk Factors for Reproductive Health, and Child Health: 2012-15).

1044 We acknowledge support from the Spanish Ministry of Science and Innovation through the  
1045 “Centro de Excelencia Severo Ochoa 2019-2023” Program (CEX2018-000806-S), and  
1046 support from the Generalitat de Catalunya through the CERCA Program.

1047 MV-U and CR-A were supported by a FI fellowship from the Catalan Government (FI-DGR  
1048 2015 and #016FI\_B 00272). MC received funding from Instituto Carlos III (Ministry of  
1049 Economy and Competitiveness) (CD12/00563 and MS16/00128).

## 1050 References

1051 Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, Irizarry  
1052 RA. 2014. Minfi: a flexible and comprehensive Bioconductor package for the analysis of  
1053 Infinium DNA methylation microarrays. *Bioinformatics* **30**:1363–9.  
1054 doi:10.1093/bioinformatics/btu049

1055 Battram T, Yousefi P, Crawford G, Prince C, Babei MS, Sharp G, Hatcher C, Vega-Salas  
1056 MJ, Khodabakhsh S, Whitehurst O, Langdon R, Mahoney L, Elliott HR, Mancano G,  
1057 Lee M, Watkins SH, Lay AC, Hemani G, Gaunt TR, Relton CL, Staley JR, Suderman M.  
1058 2021. The EWAS Catalog: a database of epigenome-wide association studies. *OSF*  
1059 *Prepr* 4. doi:10.31219/OSF.IO/837WN

1060 Bonder MJ a., Kasela S, Kals M, Tamm R, Lokk K, Barragan I, Buurman WA, Deelen P,  
1061 Greve JW, Ivanov M, Rensen SS, van Vliet-Ostaptchouk J V., Wolfs MG, Fu J, Hofker  
1062 MH, Wijmenga C, Zhernakova A, Ingelman-Sundberg M, Franke L, Milani L. 2014.  
1063 Genetic and epigenetic regulation of gene expression in fetal and adult human livers.  
1064 *BMC Genomics*. doi:10.1186/1471-2164-15-860

1065 Bonder MJ, Luijk R, Zhernakova D V, Moed M, Deelen P, Vermaat M, van Iterson M, van  
1066 Dijk F, van Galen M, Bot J, Slieker RC, Jhamai PM, Verbiest M, Suchiman HED,  
1067 Verkerk M, van der Breggen R, van Rooij J, Lakenberg N, Arindrarto W, Kielbasa SM,

- 1068 Jonkers I, van 't Hof P, Nooren I, Beekman M, Deelen J, van Heemst D, Zhernakova A,  
1069 Tigchelaar EF, Swertz MA, Hofman A, Uitterlinden AG, Pool R, van Dongen J, Hottenga  
1070 JJ, Stehouwer CDA, van der Kallen CJH, Schalkwijk CG, van den Berg LH, van Zwet  
1071 EW, Mei H, Li Y, Lemire M, Hudson TJ, Slagboom PE, Wijmenga C, Veldink JH, van  
1072 Greevenbroek MMJ, van Duijn CM, Boomsma DI, Isaacs A, Jansen R, van Meurs JBJ,  
1073 't Hoen PAC, Franke L, Heijmans BT, Heijmans BT. 2017. Disease variants alter  
1074 transcription factor levels and methylation of their binding sites. *Nat Genet* **49**:131–138.  
1075 doi:10.1038/ng.3721
- 1076 Buckberry S, Bent SJ, Bianco-Miotto T, Roberts CT. 2014. MassiR: A method for predicting  
1077 the sex of samples in gene expression microarray datasets. *Bioinformatics* **30**:2084–  
1078 2085. doi:10.1093/bioinformatics/btu161
- 1079 Cavalli G, Heard E. 2019. Advances in epigenetics link genetics to the environment and  
1080 disease. *Nature*. doi:10.1038/s41586-019-1411-0
- 1081 Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. 2015. Second-generation  
1082 PLINK: rising to the challenge of larger and richer datasets. *Gigascience* **4**:7.  
1083 doi:10.1186/s13742-015-0047-8
- 1084 Chatzi L, Leventakou V, Vafeiadi M, Koutra K, Roumeliotaki T, Chalkiadaki G, Karachaliou  
1085 M, Daraki V, Kyriklaki A, Kampouri M, Fthenou E, Sarri K, Vassilaki M, Fasoulaki M,  
1086 Bitsios P, Koutis A, Stephanou EG, Kogevinas M. 2017. Cohort Profile: The Mother-  
1087 Child Cohort in Crete, Greece (Rhea Study) **46**:1392-1393k. doi:10.1093/ije/dyx084
- 1088 Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, Vrieze SI, Chew EY, Levy S,  
1089 McGue M, Schlessinger D, Stambolian D, Loh P-R, Iacono WG, Swaroop A, Scott LJ,  
1090 Cucca F, Kronenberg F, Boehnke M, Abecasis GR, Fuchsberger C. 2016. Next-  
1091 generation genotype imputation service and methods. *Nat Genet* **48**:1284–1287.  
1092 doi:10.1038/ng.3656

- 1093 Delahaye F, Do C, Kong Y, Ashkar R, Salas M, Tycko B, Wapner R, Hughes F. 2018.  
1094 Genetic variants influence on the placenta regulatory landscape. *PLoS Genet* **14**.  
1095 doi:10.1371/journal.pgen.1007785
- 1096 Dudbridge F, Gusnanto A. 2008. Estimation of significance thresholds for genomewide  
1097 association scans. *Genet Epidemiol* **32**:227. doi:10.1002/GEPI.20297
- 1098 Feinberg AP. 2018. The Key Role of Epigenetics in Human Disease Prevention and  
1099 Mitigation. *N Engl J Med* **378**:1323–1334. doi:10.1056/nejmra1402513
- 1100 Felix JF, Joubert BR, Baccarelli AA, Sharp GC, Almqvist C, Annesi-Maesano I, Arshad H,  
1101 Baiz N, Bakermans-Kranenburg MJ, Bakulski KM, Binder EB, Bouchard L, Breton C V.,  
1102 Brunekreef B, Brunst KJ, Burchard EG, Bustamante M, Chatzi L, Munthe-Kaas MC,  
1103 Corpeleijn E, Czamara D, Dabelea D, Smith GD, De Boever P, Duijts L, Dwyer T, Eng  
1104 C, Eskenazi B, Everson TM, Falahi F, Fallin MD, Farchi S, Fernandez MF, Gao L,  
1105 Gaunt TR, Ghanous A, Gillman MW, Gonseth S, Grote V, Gruzieva O, Häberg SE,  
1106 Herceg Z, Hivert MF, Holland N, Holloway JW, Hoyo C, Hu D, Huang RC, Huen K,  
1107 Järvelin MR, Jima DD, Just AC, Karagas MR, Karlsson R, Karmaus W, Kechris KJ,  
1108 Kere J, Kogevinas M, Koletzko B, Koppelman GH, Kupers LK, Ladd-Acosta C, Lahti J,  
1109 Lambrechts N, Langie SAS, Lie RT, Liu AH, Magnus MC, Magnus P, Maguire RL,  
1110 Marsit CJ, McArdle W, Melen E, Melton P, Murphy SK, Nawrot TS, Nisticò L, Nohr EA,  
1111 Nordlund B, Nystad W, Oh SS, Oken E, Page CM, Perron P, Pershagen G, Pizzi C,  
1112 Plusquin M, Raikonen K, Reese SE, Reischl E, Richiardi L, Ring S, Roy RP, Rzehak  
1113 P, Schoeters G, Schwartz DA, Sebert S, Snieder H, Sørensen TIA, Starling AP, Sunyer  
1114 J, Taylor JA, Tiemeier H, Ullemer V, Vafeiadi M, Van Ijzendoorn MH, Vonk JM, Vriens  
1115 A, Vrijheid M, Wang P, Wiemels JL, Wilcox AJ, Wright RJ, Xu CJ, Xu Z, Yang I V.,  
1116 Yousefi P, Zhang H, Zhang W, Zhao S, Agha G, Relton CL, Jaddoe VWV, London SJ.  
1117 2018. Cohort profile: Pregnancy and childhood epigenetics (PACE) consortium. *Int J*  
1118 *Epidemiol* **47**:22-23u. doi:10.1093/ije/dyx190

- 1119 Fortin J-P, Fertig E, Hansen K. 2014a. shinyMethyl: interactive quality control of Illumina  
1120 450k DNA methylation arrays in R. *F1000Research* **3**:175.  
1121 doi:10.12688/f1000research.4680.2
- 1122 Fortin J-P, Labbe A, Lemire M, Zanke BW, Hudson TJ, Fertig EJ, Greenwood C, Hansen  
1123 KD. 2014b. Functional normalization of 450k methylation array data improves  
1124 replication in large cancer studies. *Genome Biol* **15**:503. doi:10.1186/s13059-014-0503-  
1125 2
- 1126 Fuchsberger C, Abecasis GR, Hinds DA. 2015. Minimac2: Faster genotype imputation.  
1127 *Bioinformatics* **31**:782–784. doi:10.1093/bioinformatics/btu704
- 1128 Gamazon ER, Segrè A V., Van De Bunt M, Wen X, Xi HS, Hormozdiari F, Ongen H,  
1129 Konkashbaev A, Derks EM, Aguet F, Quan J, Nicolae DL, Eskin E, Kellis M, Getz G,  
1130 McCarthy MI, Dermitzakis ET, Cox NJ, Ardlie KG. 2018. Using an atlas of gene  
1131 regulation across 44 human tissues to inform complex disease- and trait-associated  
1132 variation. *Nat Genet* **50**:956–967. doi:10.1038/s41588-018-0154-4
- 1133 Gaunt TR, Shihab HA, Hemani G, Min JL, Woodward G, Lyttleton O, Zheng J, Duggirala A,  
1134 McArdle WL, Ho K, Ring SM, Evans DM, Davey Smith G, Relton CL. 2016. Systematic  
1135 identification of genetic influences on methylation across the human life course.  
1136 *Genome Biol* **17**:61. doi:10.1186/s13059-016-0926-z
- 1137 Gondalia R, Baldassari A, Holliday KM, Justice AE, Méndez-Giráldez R, Stewart JD, Liao D,  
1138 Yanosky JD, Brennan KJM, Engel SM, Jordahl KM, Kennedy E, Ward-Caviness CK,  
1139 Wolf K, Waldenberger M, Cyrus J, Peters A, Bhatti P, Horvath S, Assimes TL, Pankow  
1140 JS, Demerath EW, Guan W, Fornage M, Bressler J, North KE, Conneely KN, Li Y, Hou  
1141 L, Baccarelli AA, Whitsel EA. 2019. Methylome-wide association study provides  
1142 evidence of particulate matter air pollution-associated DNA methylation. *Environ Int*  
1143 **132**. doi:10.1016/j.envint.2019.03.071

- 1144 Grazuleviciene R, Danileviciute A, Nadisauskiene R, Vencloviene J. 2009. Maternal  
1145 smoking, GSTM1 and GSTT1 polymorphism and susceptibility to adverse pregnancy  
1146 outcomes. *Int J Environ Res Public Health* **6**:1282–1297. doi:10.3390/ijerph6031282
- 1147 Gutierrez-Arcelus M, Lappalainen T, Montgomery SB, Buil A, Ongen H, Yurovsky A, Bryois  
1148 J, Giger T, Romano L, Planchon A, Falconnet E, Bielser D, Gagnebin M, Padioleau I,  
1149 Borel C, Letourneau A, Makrythanasis P, Guipponi M, Gehrig C, Antonarakis SE,  
1150 Dermitzakis ET. 2013. Passive and active DNA methylation and the interplay with  
1151 genetic variation in gene regulation. *Elife* **2**. doi:10.7554/eLife.00523
- 1152 Gutierrez-Arcelus M, Ongen H, Lappalainen T, Montgomery SB, Buil A, Yurovsky A, Bryois  
1153 J, Padioleau I, Romano L, Planchon A, Falconnet E, Bielser D, Gagnebin M, Giger T,  
1154 Borel C, Letourneau A, Makrythanasis P, Guipponi M, Gehrig C, Antonarakis SE,  
1155 Dermitzakis ET. 2015. Tissue-Specific Effects of Genetic and Epigenetic Variation on  
1156 Gene Regulation and Splicing. *PLoS Genet* **11**. doi:10.1371/journal.pgen.1004958
- 1157 Guxens M, Ballester F, Espada M, Fernández MF, Grimalt JO, Ibarluzea J, Olea N,  
1158 Rebagliato M, Tardón A, Torrent M, Vioque J, Vrijheid M, Sunyer J. 2012. Cohort  
1159 Profile: the INMA--INfancia y Medio Ambiente--(Environment and Childhood) Project.  
1160 *Int J Epidemiol* **41**:930–40. doi:10.1093/ije/dyr054
- 1161 Hansen K. n.d. IlluminaHumanMethylation450kanno.ilmn12.hg19: Annotation for Illumina's  
1162 450k methylation arrays.  
1163 doi:10.18129/B9.bioc.IlluminaHumanMethylation450kanno.ilmn12.hg19
- 1164 Heude B, Forhan A, Slama R, Douhaud L, Bedel S, Saurel-Cubizolles M-JJ, Hankard R,  
1165 Thiebaugeorges O, de Agostini M, Annesi-Maesano I, Kaminski M, Charles M-AA,  
1166 Annesi-Maesano I, Bernard JY, Botton J, Charles M-AA, Dargent-Molina P, de Lauzon-  
1167 Guillain B, Ducimetière P, de Agostini M, Foliguet B, Forhan A, Fritel X, Germa A, Goua  
1168 V, Hankard R, Heude B, Kaminski M, Larroque B, Lelong N, Lepeule J, Magnin G,

1169 Marchand L, Nabet C, Pierre F, Slama R, Saurel-Cubizolles M-JJ, Schweitzer M,  
1170 Thiebaugeorges O, EDEN mother-child cohort study group. 2016. Cohort Profile: The  
1171 EDEN mother-child cohort on the prenatal and early postnatal determinants of child  
1172 health and development. *Int J Epidemiol* **45**:353–363. doi:10.1093/ije/dyv151

1173 Houseman EAE, Accomando WP, Koestler DDC, Christensen BBC, Marsit CCJ, Nelson HH,  
1174 Wiencke JK, Kelsey KTK, Natoli G, Ji H, Ehrlich L, Seita J, Murakami P, Doi A, Lindau  
1175 P, Lee H, Aryee M, Irizarry R, Kim K, Rossi D, Inlay M, Serwold T, Karsunky H, Ho L,  
1176 Daley G, Weissman I, Feinberg A, Khavari D, Sen G, Rinn J, Baron U, Turbachova I,  
1177 Hellwag A, Eckhardt F, Berlin K, Hoffmuller U, Gardina P, Olek S, Wieczorek G,  
1178 Asemissen A, Model F, Turbachova I, Floess S, Liebenberg V, Baron U, Stauch D,  
1179 Kotsch K, Pratschke J, Hamann A, Loddenkemper C, Stein H, Volk H, Hoffmuller U,  
1180 Grutzkau A, Mustea A, Huehn J, Scheibenbogen C, Olek S, Sehouli J, Loddenkemper  
1181 C, Cornu T, Schwachula T, Hoffmuller U, Grutzkau A, Lohneis P, Dickhaus T, Grone J,  
1182 Kruschewski M, Mustea A, Turbachova I, Baron U, Olek S, Hanahan D, Weinberg R,  
1183 Ostrand-Rosenberg S, Lynch L, O’Connell J, Kwasnik A, Cawood T, O’Farrelly C,  
1184 O’Shea D, Anderson E, Gutierrez D, Hasty A, Chua W, Charles K, Baracos V, Clarke S,  
1185 Carroll R, Ruppert D, Stefanski L, Gaujoux R, Seoighe C, Gong T, Hartmann N,  
1186 Kohane I, Brinkmann V, Staedtler F, Letzkus M, Bongiovanni S, Szustakowski J, Shen-  
1187 Orr S, Tibshirani R, Khatri P, Bodian D, Staedtler F, Perry N, Hastie T, Sarwal M, Davis  
1188 M, Butte A, Wang S, Petronis A, Smyth G, Leek J, Storey J, Teschendorff A, Zhuang J,  
1189 Widschwendte R, Goldfarb D, Idnani A, Peters E, McClean M, Liu M, Eisen E, Mueller  
1190 N, Kelsey KTK, Teschendorff A, Menon U, Gentry-Maharaj A, Ramus S, Gayther S,  
1191 Apostolidou S, Jones A, Lechner M, Beck S, Jacobs I, Widschwendter M, Kerkel K,  
1192 Schupf N, Hatta K, Pang D, Salas M, Kratz A, Minden M, Murty V, Zigman W, Mayeux  
1193 R, Jenkins E, Torkamani A, Schork N, Silverman W, Croy B, Tycko B, Wang X, Zhu H,  
1194 Snieder H, Su S, Munn D, Harshfield G, Maria B, Dong Y, Treiber F, Gutin B, Shi H,  
1195 Trellakis S, Bruderek K, Dumitru C, Gholaman H, Gu X, Bankfalvi A, Scherag A, Hutte

1196 J, Dominas N, Lehnerdt G, Hoffmann T, Lang S, Brandau S, Kuss I, Hathaway B, Ferris  
1197 R, Gooding W, Whiteside T, Kuss I, Hathaway B, Ferris R, Gooding W, Whiteside T,  
1198 Mold J, Venkatasubrahmanyam S, Burt T, Michaelsson J, Rivera J, Galkina S,  
1199 Weinberg K, Stoddart C, McCune J, Ouden M den, Ubachs J, Stoot J, Wersch J van,  
1200 Bishara S, Griffin M, Cargill A, Bali A, Gore M, Kaye S, Shepherd J, Trappen P Van,  
1201 Cho H, Hur H, Kim SS, Kim SS, Kim J, Kim Y, Lee K, Verstegen R, Kusters M, Gemen  
1202 E, Vries E De, Ram G, Chinen J, Thurston S, Spiegelman D, Ruppert D, Li B, Yin X,  
1203 Goeman J, Buhlmann P, Subramanian A, Tamayo P, Mootha V, Mukherjeed S, Ebert  
1204 B, Gillette M, Paulovich A, Pomeroy S, Golub T, Lander E, Mesirov J, Carroll R, Galindo  
1205 C, Little R, Rubin D, Koestler DDC, Marsit CCJ, Christensen BBC, Karagas M, Bueno  
1206 R, Sugarbaker D, Kelsey KTK, Houseman EAE, Marsit CCJ, Koestler DDC,  
1207 Christensen BBC, Karagas M, Houseman EAE, Kelsey KTK, Pedersen K, Bamlet W,  
1208 Oberg A, Andrade M de, Matsumoto M, Tang H, Thibodeau S, Petersen G, Wang L,  
1209 Bocklandt S, Lin W, Sehl M, Sanchez F, Sinsheimer J, Horvath S, Vilain E, Chu M,  
1210 Siegmund K, Hao Q, Crooks G, Tavaré S, Shibata D, Doi A, Park I, Wen B, Murakami  
1211 P, Aryee M, Houseman EAE, Christensen BBC, Yeh R, Marsit CCJ, Karagas M, Alberts  
1212 B, Johnson A, Lewis J, Raff M, Roberts K, Showe M, Vachani A, Kossenkov A, Yousef  
1213 M, Nichols C, Kossenkov A, Vachani A, Chang C, Nichols C, Billouin S, Watkins N,  
1214 Gusnanto A, Bono B de, De S, Miranda-Saavedra D, Ginns L, Goldenheim P, Miller L,  
1215 Burton R, Gillick L, Mazzocchi G, Balzanelli M, Giuliani A, Cata A De, Viola M La. 2012.  
1216 DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC*  
1217 *Bioinformatics* **13**:86. doi:10.1186/1471-2105-13-86

1218 Huse SM, Gruppuso PA, Boekelheide K, Sanders JA. 2015. Patterns of gene expression  
1219 and DNA methylation in human fetal and adult liver. *BMC Genomics* **16**:981.  
1220 doi:10.1186/s12864-015-2066-3

1221 Husquin LT, Rotival M, Fagny M, Quach H, Zidane N, McEwen LM, MacIsaac JL, Kobor MS,  
1222 Aschard H, Patin E, Quintana-Murci L. 2018. Exploring the genetic basis of human



- 1223 population differences in DNA methylation and their causal impact on immune gene  
1224 regulation 06 Biological Sciences 0604 Genetics. *Genome Biol* **19**. doi:10.1186/s13059-  
1225 018-1601-3
- 1226 J AA and R. 2010. topGO: topGO: Enrichment analysis for Gene Ontology. No Title.
- 1227 Johnson ND, Wiener HW, Smith AK, Nishitani S, Absher DM, Arnett DK, Aslibekyan S,  
1228 Conneely KN. 2017. Non-linear patterns in age-related DNA methylation may reflect  
1229 CD4+ T cell differentiation. *Epigenetics* **12**:492–503.  
1230 doi:10.1080/15592294.2017.1314419
- 1231 Johnson WE, Li C, Rabinovic A. 2007. Adjusting batch effects in microarray expression data  
1232 using empirical Bayes methods. *Biostatistics* **8**:118–27. doi:10.1093/biostatistics/kxj037
- 1233 Jones PA. 2012. Functions of DNA methylation: Islands, start sites, gene bodies and  
1234 beyond. *Nat Rev Genet*. doi:10.1038/nrg3230
- 1235 Kennedy EM, Goehring GN, Nichols MH, Robins C, Mehta D, Klengel T, Eskin E, Smith AK,  
1236 Conneely KN. 2018. An integrated -omics analysis of the epigenetic landscape of gene  
1237 expression in human blood cells. *BMC Genomics* **19**. doi:10.1186/s12864-018-4842-3
- 1238 Kim S, Forno E, Zhang R, Park HJ, Xu Z, Yan Q, Boutaoui N, Acosta-Pérez E, Canino G,  
1239 Chen W, Celedón JC. 2020. Expression Quantitative Trait Methylation Analysis Reveals  
1240 Methylomic Associations With Gene Expression in Childhood Asthma. *Chest*.  
1241 doi:10.1016/j.chest.2020.05.601
- 1242 Küpers LK, Monnereau C, Sharp GC, Yousefi P, Salas LA, Ghantous A, Page CM, Reese  
1243 SE, Wilcox AJ, Czamara D, Starling AP, Novoloaca A, Lent S, Roy R, Hoyo C, Breton C  
1244 V., Allard C, Just AC, Bakulski KM, Holloway JW, Everson TM, Xu CJ, Huang RC, van  
1245 der Plaats DA, Wielscher M, Merid SK, Ullemer V, Rezwan FI, Lahti J, van Dongen J,  
1246 Langie SAS, Richardson TG, Magnus MC, Nohr EA, Xu Z, Duijts L, Zhao S, Zhang W,

1247 Plusquin M, DeMeo DL, Solomon O, Heimovaara JH, Jima DD, Gao L, Bustamante M,  
1248 Perron P, Wright RO, Hertz-Picciotto I, Zhang H, Karagas MR, Gehring U, Marsit CJ,  
1249 Beilin LJ, Vonk JM, Jarvelin MR, Bergström A, Örtqvist AK, Ewart S, Villa PM, Moore  
1250 SE, Willemsen G, Standaert ARL, Håberg SE, Sørensen TIA, Taylor JA, Räikkönen K,  
1251 Yang I V., Kechris K, Nawrot TS, Silver MJ, Gong YY, Richiardi L, Kogevinas M,  
1252 Litonjua AA, Eskenazi B, Huen K, Mbarek H, Maguire RL, Dwyer T, Vrijheid M,  
1253 Bouchard L, Baccarelli AA, Croen LA, Karmaus W, Anderson D, de Vries M, Sebert S,  
1254 Kere J, Karlsson R, Arshad SH, Hämäläinen E, Routledge MN, Boomsma DI, Feinberg  
1255 AP, Newschaffer CJ, Govarts E, Moisse M, Fallin MD, Melén E, Prentice AM, Kajantie  
1256 E, Almqvist C, Oken E, Dabelea D, Boezen HM, Melton PE, Wright RJ, Koppelman GH,  
1257 Trevisi L, Hivert MF, Sunyer J, Munthe-Kaas MC, Murphy SK, Corpeleijn E, Wiemels J,  
1258 Holland N, Herceg Z, Binder EB, Davey Smith G, Jaddoe VVW, Lie RT, Nystad W,  
1259 London SJ, Lawlor DA, Relton CL, Snieder H, Felix JF. 2019. Meta-analysis of  
1260 epigenome-wide association studies in neonates reveals widespread differential DNA  
1261 methylation associated with birthweight. *Nat Commun* **10**. doi:10.1038/s41467-019-  
1262 09671-3

1263 Lappalainen T, Grealley JM. 2017. Associating cellular epigenetic models with human  
1264 phenotypes. *Nat Rev Genet*. doi:10.1038/nrg.2017.32

1265 Leek JT, Storey JD, Qiu X, Xiao Y, Gordon A, Yakovlev A, Klebanov L, Yakovlev A, Kerr M,  
1266 Martin M, Churchill G, Kerr M, Churchill G, Holter N, Mitra M, Maritan A, Cieplak M,  
1267 Banavar J, Gasch A, Spellman P, Kao C, Carmel-Harel O, Eisen M, Rodwell G, Sonu  
1268 R, Zahn J, Lund J, Wilhelmy J, Storey J, Xiao W, T L, Tompkins R, Davis R, DeRisi J,  
1269 Iyer V, Brown P, Brem R, Yvert G, Clinton R, Kruglyak L, Schadt E, Monks S, Drake T,  
1270 Lusi A, Che N, Tseng G, Oh M, Rohlin L, Liao J, Wong W, Yang Y, Dudoit S, Luu P,  
1271 Lin D, Peng V, Qui X, Klebanov L, Yakovlev A, Morley M, Molony C, Weber T, Devlin J,  
1272 Ewens K, Rhodes D, Chinnaiyan A, Nguyen D, Sam K, Tsimelzon A, Li X, Wong H,  
1273 Amundson S, Bittner M, Chen Y, Trent J, Meltzer P, Lamb J, Crawford E, Peck D,

- 1274 Modell J, Blat I, Dabney A, Storey J, Brem R, Storey J, Whittle J, Kruglyak L, Hedenfalk  
1275 I, Duggan D, Chen Y, Radmacher M, Bittner M, Storey J, Tibshirani R, Dabney A,  
1276 Storey J, Rice J, Storey J, Buja A, Eyuboglu N, Lehman E, Romano J, Owen A, Qiu X,  
1277 Yakovlev A, Efron B, Efron B, Cai G, Sarkar S, Benjamini Y, Yekutieli D, Pawitan Y,  
1278 Calza S, Ploner A, Yvert G, Brem R, Whittle J, Akey J, Foss E, Eisen M, Spellman P,  
1279 Brown P, Botstein D, Hedenfalk I, Ringer M, Ben-Dor A, Yakhini Z, Chen Y, Mardia K,  
1280 Kent J, Bibby J, Alter O, Brown P, Botstein D, Price A, Patterson N, Plenge R, Weinblatt  
1281 M, SN A, Storey J, Akey J, Kruglyak L, Hastie T, Tibshirani R. 2007. Capturing  
1282 heterogeneity in gene expression studies by surrogate variable analysis. *PLoS Genet*  
1283 **3**:1724–1735. doi:10.1371/journal.pgen.0030161
- 1284 Lehne B, Drong AW, Loh M, Zhang W, Scott WR, Tan S-T, Afzal U, Scott J, Jarvelin M-R,  
1285 Elliott P, McCarthy MI, Kooner JS, Chambers JC. 2015. A coherent approach for  
1286 analysis of the Illumina HumanMethylation450 BeadChip improves data quality and  
1287 performance in epigenome-wide association studies. *Genome Biol* **16**:37.  
1288 doi:10.1186/s13059-015-0600-x
- 1289 Leland Taylor D, Jackson AU, Narisu N, Hemani G, Erdos MR, Chines PS, Swift A, Idol J,  
1290 Didion JP, Welch RP, Kinnunen L, Saramies J, Lakka TA, Laakso M, Tuomilehto J,  
1291 Parker SCJ, Koistinen HA, Smith GD, Boehnke M, Scott LJ, Birney E, Collins FS. 2019.  
1292 Integrative analysis of gene expression, DNA methylation, physiological traits, and  
1293 genetic variation in human skeletal muscle. *Proc Natl Acad Sci U S A* **166**:10883–  
1294 10888. doi:10.1073/pnas.1814263116
- 1295 Li M, Zou D, Li Z, Gao R, Sang J, Zhang Y, Li R, Xia L, Zhang T, Niu G, Bao Y, Zhang Z.  
1296 2019. EWAS Atlas: A curated knowledgebase of epigenome-wide association studies.  
1297 *Nucleic Acids Res* **47**:D983–D988. doi:10.1093/nar/gky1027
- 1298 Lin X, Teh AL, Chen L, Lim IY, Tan PF, Maclsaac JL, Morin AM, Yap F, Tan KH, Saw SM,  
1299 Lee YS, Holbrook JD, Godfrey KM, Meaney MJ, Kobor MS, Chong YS, Gluckman PD,

- 1300 Karnani N. 2017. Choice of surrogate tissue influences neonatal EWAS findings. *BMC*  
1301 *Med* **15**. doi:10.1186/s12916-017-0970-x
- 1302 Liu Y, Ding J, Reynolds LM, Lohman K, Register TC, De la Fuente A, Howard TD, Hawkins  
1303 GA, Cui W, Morris J, Smith SG, Barr RG, Kaufman JD, Burke GL, Post W, Shea S,  
1304 McCall CE, Siscovick D, Jacobs DR, Tracy RP, Herrington DM, Hoeschele I. 2013.  
1305 Methyloomics of gene expression in human monocytes. *Hum Mol Genet* **22**:5065–5074.  
1306 doi:10.1093/hmg/ddt356
- 1307 Loh PR, Danecek P, Palamara PF, Fuchsberger C, Reshef YA, Finucane HK, Schoenherr S,  
1308 Forer L, McCarthy S, Abecasis GR, Durbin R, Price AL. 2016. Reference-based  
1309 phasing using the Haplotype Reference Consortium panel. *Nat Genet* **48**:1443–1448.  
1310 doi:10.1038/ng.3679
- 1311 Lu Y, Wang B, Jiang F, Mo X, Wu L, He P, Lu X, Deng F, Lei S. 2019. Multi-omics  
1312 integrative analysis identified SNP-methylation-mRNA: Interaction in peripheral blood  
1313 mononuclear cells. *J Cell Mol Med* **23**:4601. doi:10.1111/JCMM.14315
- 1314 Magnus P, Birke C, Vejrup K, Haugan A, Alsaker E, Daltveit AK, Handal M, Haugen M,  
1315 Høiseth G, Knudsen GP, Paltiel L, Schreuder P, Tambs K, Vold L, Stoltenberg C. 2016.  
1316 Cohort Profile Update: The Norwegian Mother and Child Cohort Study (MoBa). *Int J*  
1317 *Epidemiol* **45**:382–388. doi:10.1093/ije/dyw029
- 1318 Maitre L, De Bont J, Casas M, Robinson O, Aasvang GM, Agier L, Andrušaitytė S, Ballester  
1319 F, Basagaña X, Borràs E, Brochet C, Bustamante M, Carracedo A, De Castro M,  
1320 Dedele A, Donaire-Gonzalez D, Estivill X, Evandt J, Fossati S, Giorgis-Allemand L,  
1321 Gonzalez JR, Granum B, Grazuleviciene R, Gützkow KB, Haug LS, Hernandez-Ferrer  
1322 C, Heude B, Ibarluzea J, Julvez J, Karachaliou M, Keun HC, Krog NH, Lau CHE,  
1323 Leventakou V, Lyon-Caen S, Manzano C, Mason D, McEachan R, Meltzer HM,  
1324 Petraviciene I, Quentin J, Roumeliotaki T, Sabido E, Saulnier PJ, Siskos AP, Siroux V,

- 1325 Sunyer J, Tamayo I, Urquiza J, Vafeiadi M, Van Gent D, Vives-Usano M, Waiblinger D,  
1326 Warembourg C, Chatzi L, Coen M, Van Den Hazel P, Nieuwenhuijsen MJ, Slama R,  
1327 Thomsen C, Wright J, Vrijheid M. 2018. Human Early Life Exposome (HELIX) study: A  
1328 European population-based exposome cohort. *BMJ Open* **8**. doi:10.1136/bmjopen-  
1329 2017-021311
- 1330 McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, Kang HM,  
1331 Fuchsberger C, Danecek P, Sharp K, Luo Y, Sidore C, Kwong A, Timpson N, Koskinen  
1332 S, Vrieze S, Scott LJ, Zhang H, Mahajan A, Veldink J, Peters U, Pato C, van Duijn CM,  
1333 Gillies CE, Gandin I, Mezzavilla M, Gilly A, Cocca M, Traglia M, Angius A, Barrett JC,  
1334 Boomsma D, Branham K, Breen G, Brummett CM, Busonero F, Campbell H, Chan A,  
1335 Chen S, Chew E, Collins FS, Corbin LJ, Smith GD, Dedoussis G, Dorr M, Farmaki A-E,  
1336 Ferrucci L, Forer L, Fraser RM, Gabriel S, Levy S, Groop L, Harrison T, Hattersley A,  
1337 Holmen OL, Hveem K, Kretzler M, Lee JC, McGue M, Meitinger T, Melzer D, Min JL,  
1338 Mohlke KL, Vincent JB, Nauck M, Nickerson D, Palotie A, Pato M, Pirastu N, McInnis  
1339 M, Richards JB, Sala C, Salomaa V, Schlessinger D, Schoenherr S, Slagboom PE,  
1340 Small K, Spector T, Stambolian D, Tuke M, Tuomilehto J, Van den Berg LH, Van  
1341 Rheenen W, Volker U, Wijmenga C, Toniolo D, Zeggini E, Gasparini P, Sampson MG,  
1342 Wilson JF, Frayling T, de Bakker PIW, Swertz MA, McCarroll S, Kooperberg C, Dekker  
1343 A, Altshuler D, Willer C, Iacono W, Ripatti S, Soranzo N, Walter K, Swaroop A, Cucca  
1344 F, Anderson CA, Myers RM, Boehnke M, McCarthy MI, Durbin R, Abecasis G, Marchini  
1345 J. 2016. A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet*  
1346 **48**:1279–1283. doi:10.1038/ng.3643
- 1347 Melé M, Ferreira PG, Reverter F, DeLuca DS, Monlong J, Sammeth M, Young TR,  
1348 Goldmann JM, Pervouchine DD, Sullivan TJ, Johnson R, Segrè A V., Djebali S,  
1349 Niarchou A, Wright FA, Lappalainen T, Calvo M, Getz G, Dermitzakis ET, Ardlie KG,  
1350 Guigó R. 2015. The human transcriptome across tissues and individuals. *Science (80- )*  
1351 **348**:660–665. doi:10.1126/science.aaa0355

- 1352 Pedersen BS, Quinlan AR. 2017. Who's Who? Detecting and Resolving Sample Anomalies  
1353 in Human DNA Sequencing Studies with Peddy. *Am J Hum Genet* **100**:406–413.  
1354 doi:10.1016/j.ajhg.2017.01.017
- 1355 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de  
1356 Bakker PIW, Daly MJ, Sham PC. 2007. PLINK: A Tool Set for Whole-Genome  
1357 Association and Population-Based Linkage Analyses. *Am J Hum Genet* **81**:559–575.  
1358 doi:10.1086/519795
- 1359 Reinius LE, Acevedo N, Joerink M, Pershagen G, Dahlén SE, Greco D, Söderhäll C,  
1360 Scheynius A, Kere J. 2012. Differential DNA methylation in purified human blood cells:  
1361 Implications for cell lineage and studies on disease susceptibility. *PLoS One* **7**:e41361.  
1362 doi:10.1371/journal.pone.0041361
- 1363 RH M, A N, CAM C, E W, LC H, AJ S, J R, BT H, TR G, JF F, VWV J, MJ B-K, H T, CL R,  
1364 MH van Ij, M S. 2021. Epigenome-wide change and variation in DNA methylation in  
1365 childhood: trajectories from birth to late adolescence. *Hum Mol Genet* **30**:119–134.  
1366 doi:10.1093/HMG/DDAA280
- 1367 Roadmap Epigenomics Consortium RE, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen  
1368 A, Heravi-Moussavi A, Kheradpour P, Zhang Z, Wang J, Ziller MJ, Amin V, Whitaker  
1369 JW, Schultz MD, Ward LD, Sarkar A, Quon G, Sandstrom RS, Eaton ML, Wu Y-C,  
1370 Pfenning AR, Wang X, Claussnitzer M, Liu Y, Coarfa C, Harris RA, Shores N, Epstein  
1371 CB, Gjoneska E, Leung D, Xie W, Hawkins RD, Lister R, Hong C, Gascard P, Mungall  
1372 AJ, Moore R, Chuah E, Tam A, Canfield TK, Hansen RS, Kaul R, Sabo PJ, Bansal MS,  
1373 Carles A, Dixon JR, Farh K-H, Feizi S, Karlic R, Kim A-R, Kulkarni A, Li D, Lowdon R,  
1374 Elliott G, Mercer TR, Neph SJ, Onuchic V, Polak P, Rajagopal N, Ray P, Sallari RC,  
1375 Siebenthall KT, Sinnott-Armstrong NA, Stevens M, Thurman RE, Wu J, Zhang B, Zhou  
1376 X, Beaudet AE, Boyer LA, De Jager PL, Farnham PJ, Fisher SJ, Haussler D, Jones  
1377 SJM, Li W, Marra MA, McManus MT, Sunyaev S, Thomson JA, Tlsty TD, Tsai L-H,

- 1378 Wang W, Waterland RA, Zhang MQ, Chadwick LH, Bernstein BE, Costello JF, Ecker  
1379 JR, Hirst M, Meissner A, Milosavljevic A, Ren B, Stamatoyannopoulos JA, Wang T,  
1380 Kellis M. 2015. Integrative analysis of 111 reference human epigenomes. *Nature*  
1381 **518**:317–30. doi:10.1038/nature14248
- 1382 Shabalin AA. 2012. Matrix eQTL: Ultra fast eQTL analysis via large matrix operations,  
1383 *Bioinformatics*. *Bioinformatics*. doi:10.1093/bioinformatics/bts163
- 1384 Sharp GC, Salas LA, Monnereau C, Allard C, Yousefi P, Everson TM, Bohlin J, Xu Z, Huang  
1385 RC, Reese SE, Xu CJ, Baiz N, Hoyo C, Agha G, Roy R, Holloway JW, Ghantous A,  
1386 Merid SK, Bakulski KM, Küpers LK, Zhang H, Richmond RC, Page CM, Duijts L, Lie  
1387 RT, Melton PE, Vonk JM, Nohr EA, Williams-DeVane CL, Huen K, Rifas-Shiman SL,  
1388 Ruiz-Arenas C, Gonseth S, Rezwan FI, Herceg Z, Ekström S, Croen L, Falahi F, Perron  
1389 P, Karagas MR, Quraishi BM, Suderman M, Magnus MC, Jaddoe VVW, Taylor JA,  
1390 Anderson D, Zhao S, Smit HA, Josey MJ, Bradman A, Baccarelli AA, Bustamante M,  
1391 Håberg SE, Pershagen G, Hertz-Picciotto I, Newschaffer C, Corpeleijn E, Bouchard L,  
1392 Lawlor DA, Maguire RL, Barcellos LF, Smith GD, Eskenazi B, Karmaus W, Marsit CJ,  
1393 Hivert MF, Snieder H, Fallin MD, Melén E, Munthe-Kaas MC, Arshad H, Wiemels JL,  
1394 Annesi-Maesano I, Vrijheid M, Oken E, Holland N, Murphy SK, Sørensen TIA,  
1395 Koppelman GH, Newnham JP, Wilcox AJ, Nystad W, London SJ, Felix JF, Relton CL.  
1396 2017. Maternal BMI at the start of pregnancy and offspring epigenome-wide DNA  
1397 methylation: Findings from the pregnancy and childhood epigenetics (PACE)  
1398 consortium. *Hum Mol Genet* **26**:4067–4085. doi:10.1093/hmg/ddx290
- 1399 Sugden K, Hannon EJ, Arseneault L, Belsky DW, Corcoran DL, Fisher HL, Houts RM,  
1400 Kandaswamy R, Moffitt TE, Poulton R, Prinz JA, Rasmussen LJH, Williams BS, Wong  
1401 CCY, Mill J, Caspi A. 2020. Patterns of Reliability: Assessing the Reproducibility and  
1402 Integrity of DNA Methylation Measurement. *Patterns* **1**:100014.  
1403 doi:10.1016/j.patter.2020.100014

- 1404 Tsai PC, Glastonbury CA, Eliot MN, Bollepalli S, Yet I, Castillo-Fernandez JE, Carnero-  
1405 Montoro E, Hardiman T, Martin TC, Vickers A, Mangino M, Ward K, Pietiläinen KH,  
1406 Deloukas P, Spector TD, Viñuela A, Loucks EB, Ollikainen M, Kelsey KT, Small KS,  
1407 Bell JT. 2018. Smoking induces coordinated DNA methylation and gene expression  
1408 changes in adipose tissue with consequences for metabolic health 06 Biological  
1409 Sciences 0604 Genetics. *Clin Epigenetics* **10**. doi:10.1186/s13148-018-0558-0
- 1410 van Dongen J, Nivard MG, Willemsen G, Hottenga J-J, Helmer Q, Dolan C V., Ehli EA,  
1411 Davies GE, van Ijzerman M, Breeze CE, Beck S, Hoen PAC', Pool R, van Greevenbroek  
1412 MMJ, Stehouwer CDA, Kallen CJH van der, Schalkwijk CG, Wijmenga C, Zernakova  
1413 S, Tigchelaar EF, Beekman M, Deelen J, van Heemst D, Veldink JH, van den Berg LH,  
1414 van Duijn CM, Hofman BA, Uitterlinden AG, Jhamai PM, Verbiest M, Verkerk M, van  
1415 der Breggen R, van Rooij J, Lakenberg N, Mei H, Bot J, Zernakova D V., van't Hof P,  
1416 Deelen P, Nooren I, Moed M, Vermaat M, Luijk R, Bonder MJ, van Dijk F, van Galen M,  
1417 Arindrarto W, Kielbasa SM, Swertz MA, van Zwet EW, Isaacs A, Franke L, Suchiman  
1418 HE, Jansen R, van Meurs JB, Heijmans BT, Slagboom PE, Boomsma DI. 2016.  
1419 Genetic and environmental influences interact with age and sex in shaping the human  
1420 methylome. *Nat Commun* **7**:11115. doi:10.1038/ncomms11115
- 1421 van Ijzerman M, Tobi EW, Slieker RC, den Hollander W, Luijk R, Slagboom PE, Heijmans BT.  
1422 2014. MethylAid: Visual and interactive quality control of large Illumina 450k data sets.  
1423 *Bioinformatics* **30**:3435–3437. doi:10.1093/bioinformatics/btu566
- 1424 Wagner JR, Busche S, Ge B, Kwan T, Pastinen T, Blanchette M. 2014. The relationship  
1425 between DNA methylation, genetic and expression inter-individual variation in  
1426 untransformed human fibroblasts. *Genome Biol* **15**. doi:10.1186/gb-2014-15-2-r37
- 1427 Wright J, Small N, Raynor P, Tuffnell D, Bhopal R, Cameron N, Fairley L, A Lawlor D,  
1428 Parslow R, Petherick ES, Pickett KE, Waiblinger D, West J. 2013. Cohort profile: The  
1429 born in bradford multi-ethnic family cohort study. *Int J Epidemiol* **42**:978–991.



1430 doi:10.1093/ije/dys112

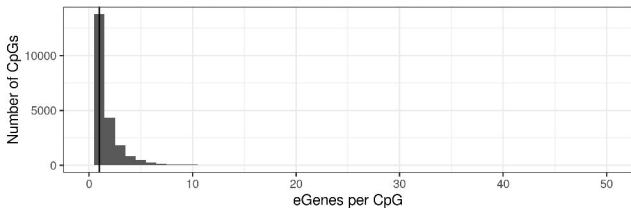
1431 Wu Y, Zeng J, Zhang F, Zhu Z, Qi T, Zheng Z, Lloyd-Jones LR, Marioni RE, Martin NG,  
1432 Montgomery GW, Deary IJ, Wray NR, Visscher PM, McRae AF, Yang J. 2018.  
1433 Integrative analysis of omics summary data reveals putative mechanisms underlying  
1434 complex traits. *Nat Commun* **9**. doi:10.1038/s41467-018-03371-0

1435 Xu C-J, Bonder MJ, Söderhäll C, Bustamante M, Baiz N, Gehring U, Jankipersadsing SA,  
1436 van der Vlies P, van Diemen CC, van Rijkom B, Just J, Kull I, Kere J, Antó JM,  
1437 Bousquet J, Zhernakova A, Wijmenga C, Annesi-Maesano I, Sunyer J, Melén E, Li Y,  
1438 Postma DS, Koppelman GH. 2017. The emerging landscape of dynamic DNA  
1439 methylation in early childhood. *BMC Genomics* **18**:25. doi:10.1186/s12864-016-3452-1

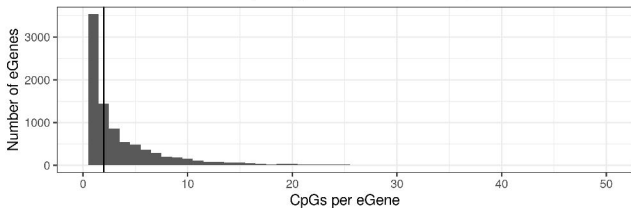
1440

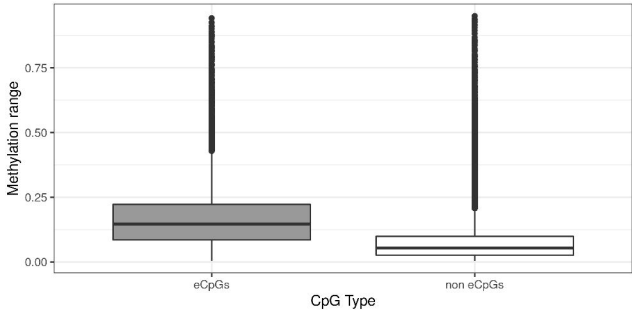
**A**

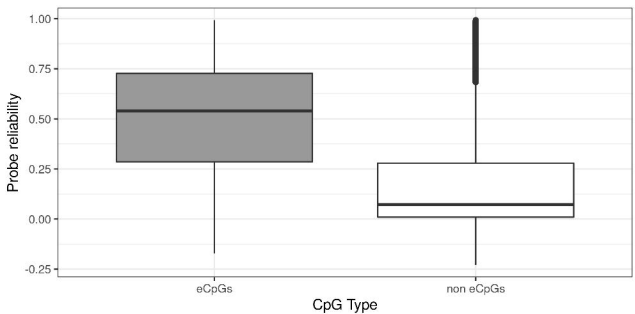
CpGs pairing distribution in cis eQTMs

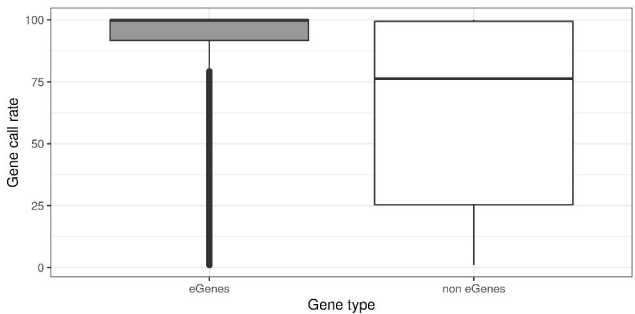
**B**

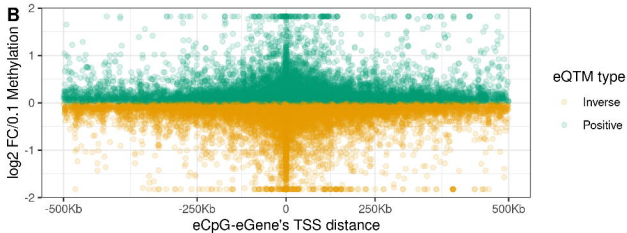
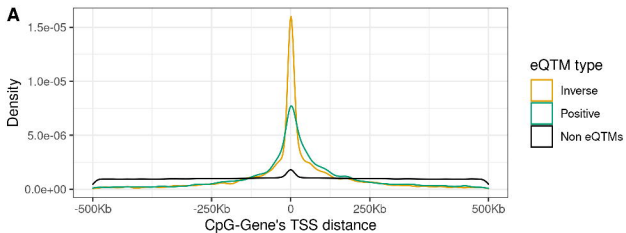
eGenes pairing distribution in cis eQTMs

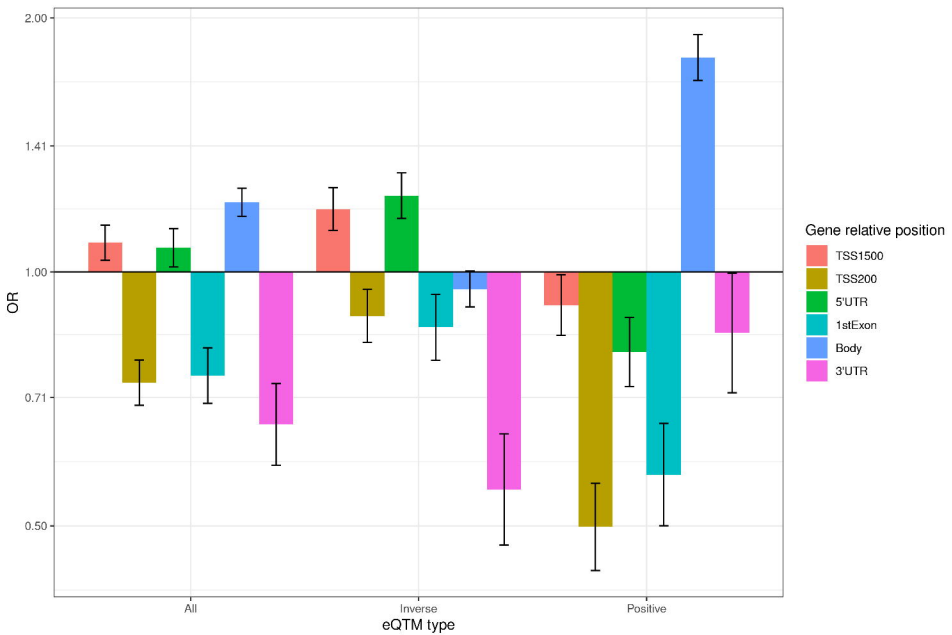




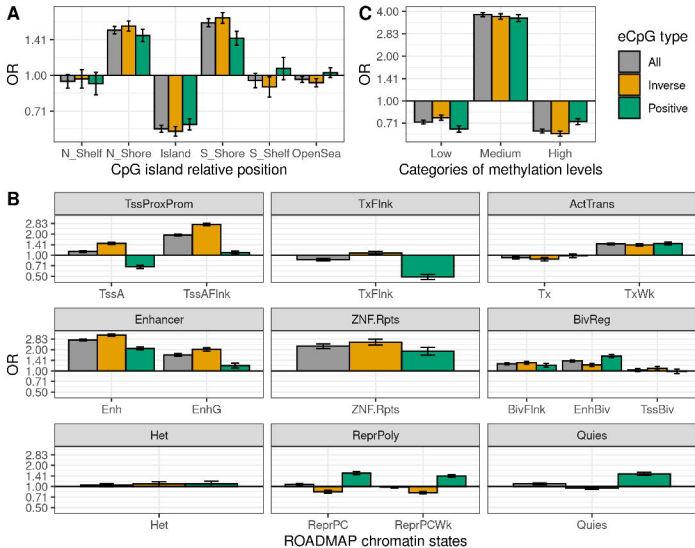




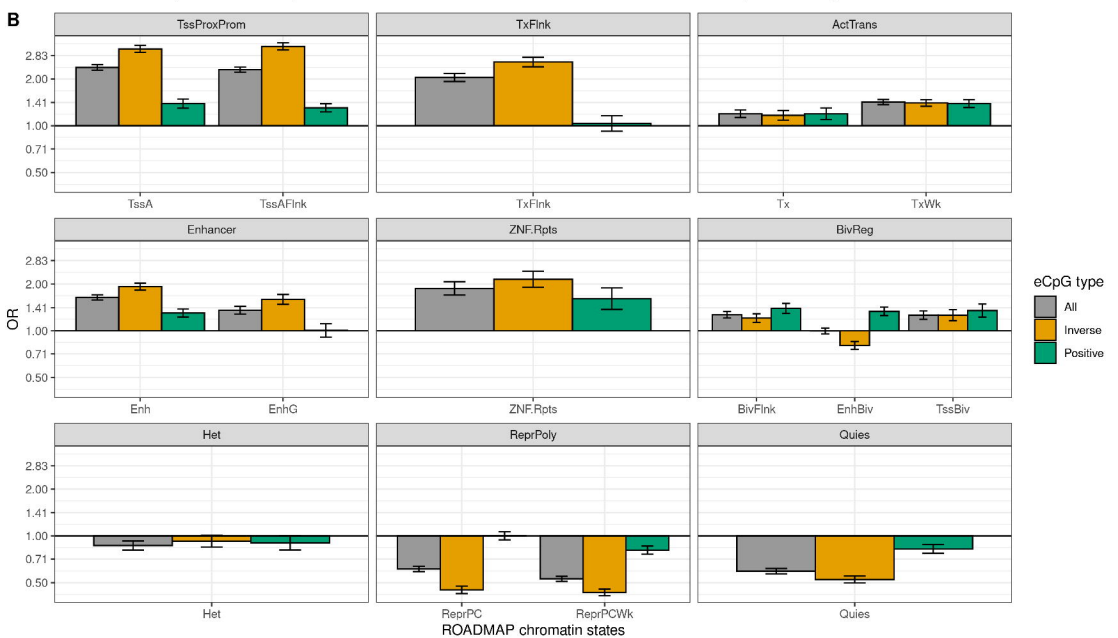
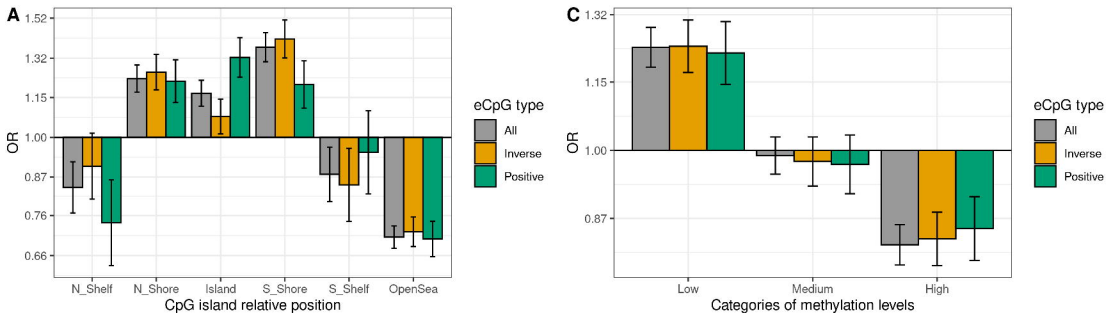


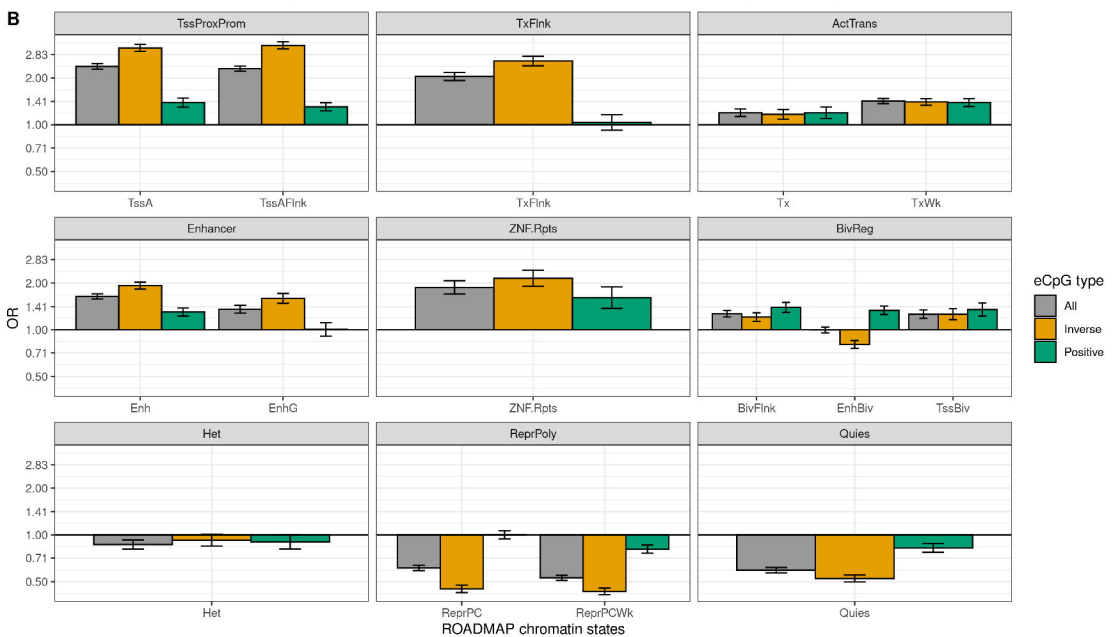
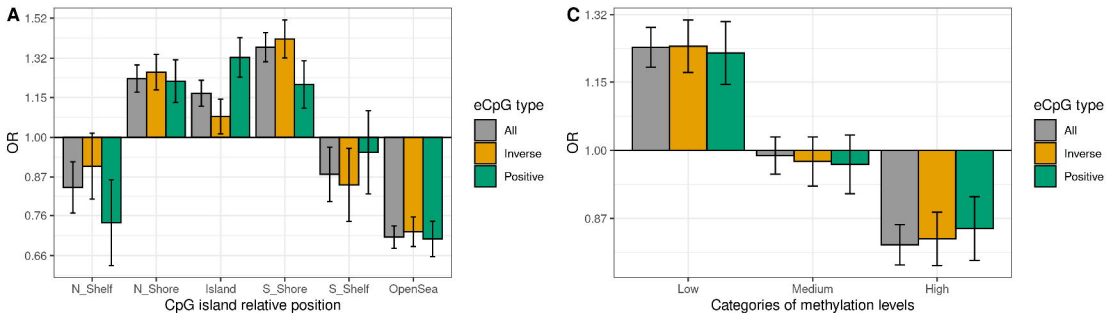


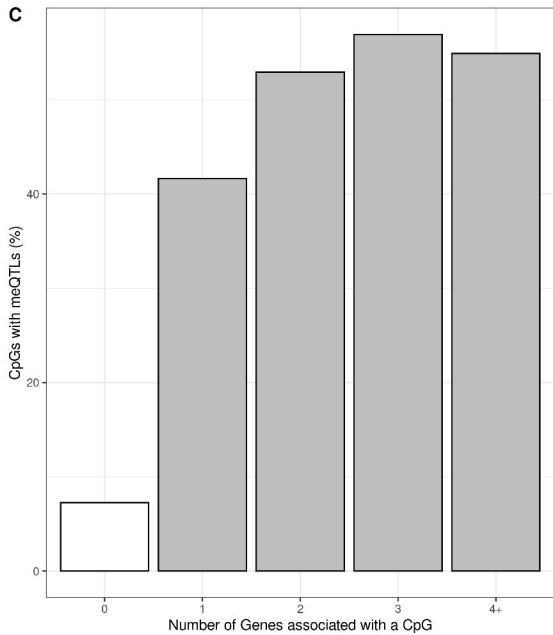
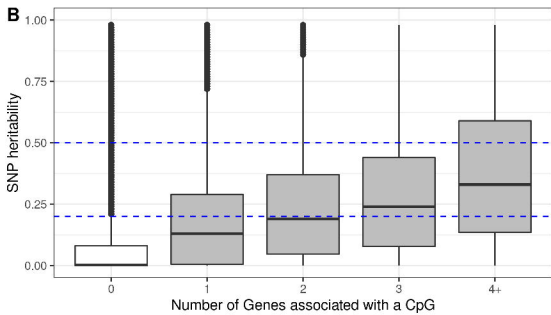
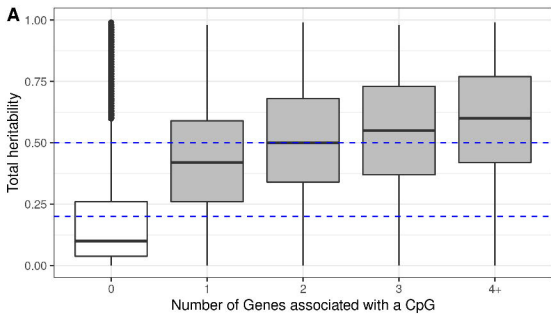
# Enrichment for regulatory elements

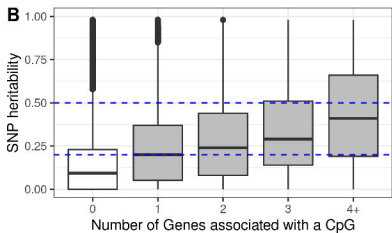
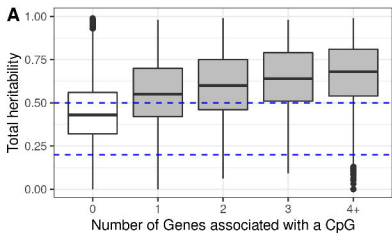


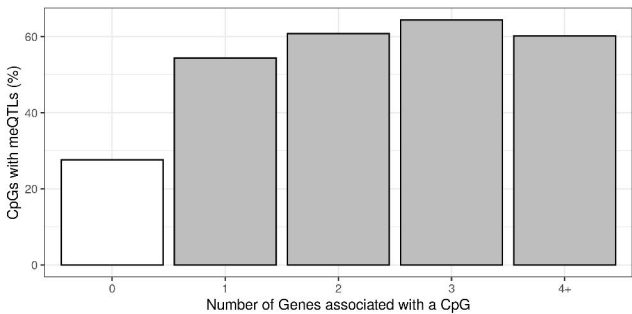


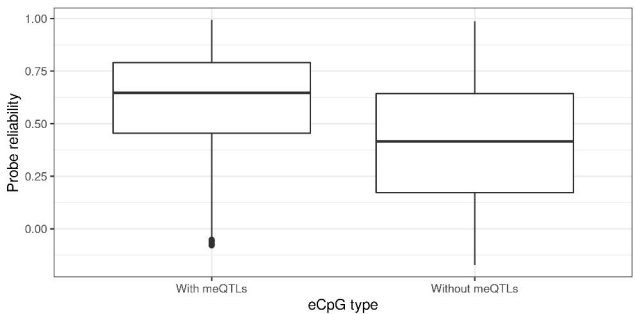


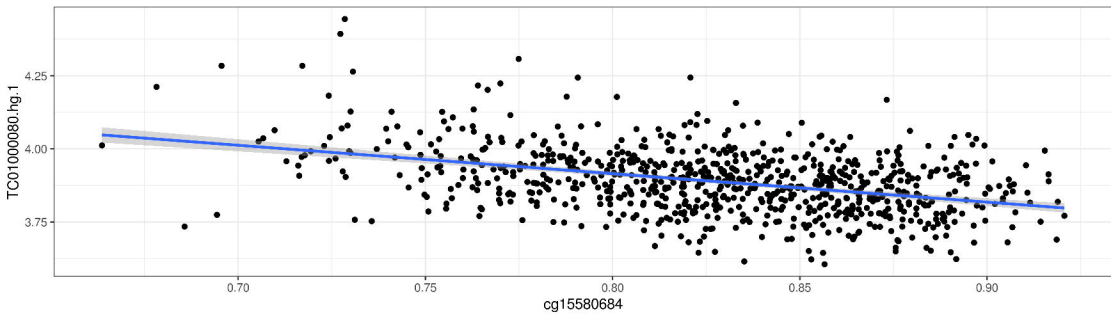
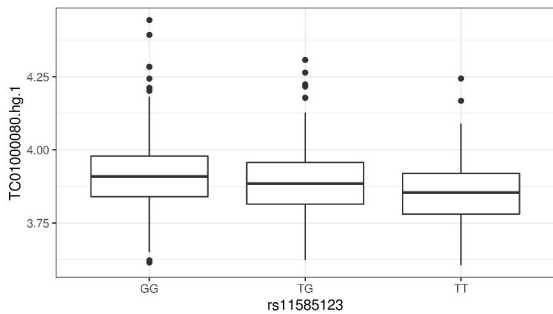
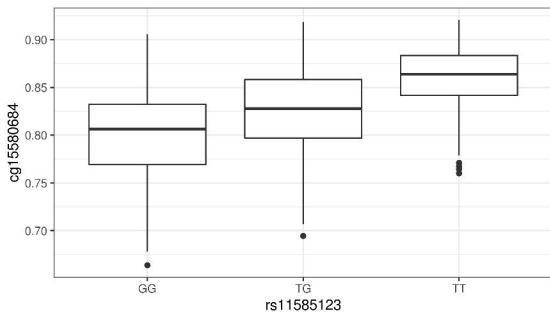


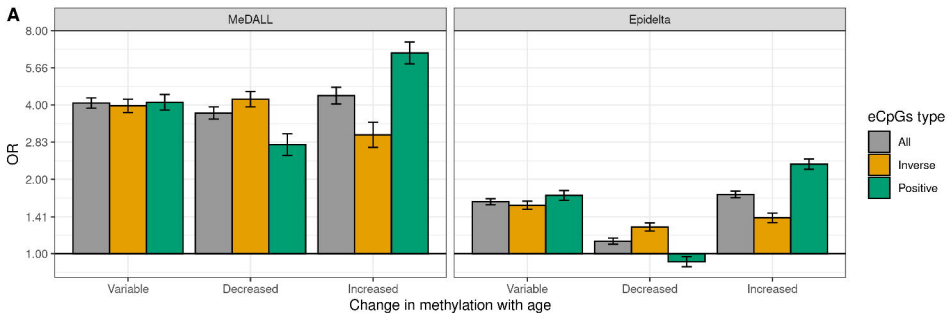




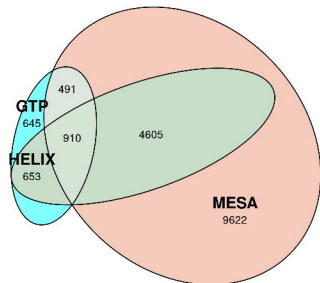




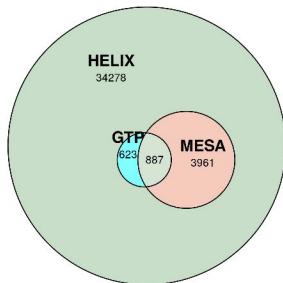




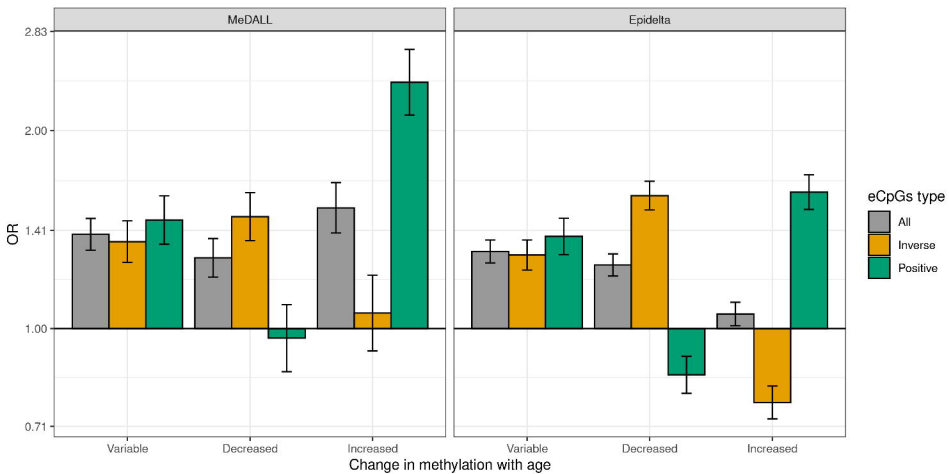
**B** Adult eQTM in HELIX

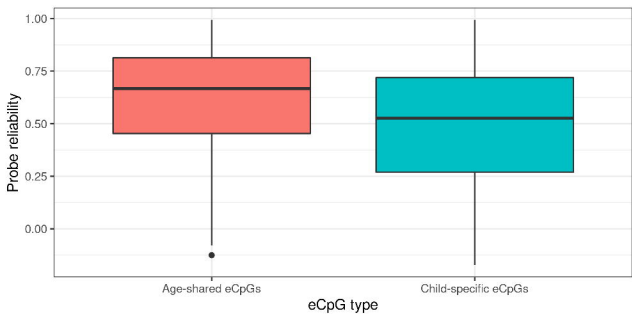


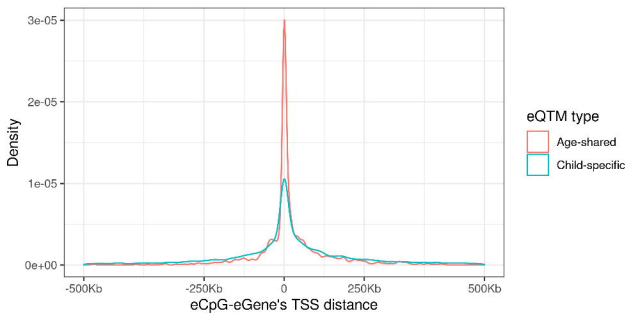
**c** HELIX eQTM in adult cohorts

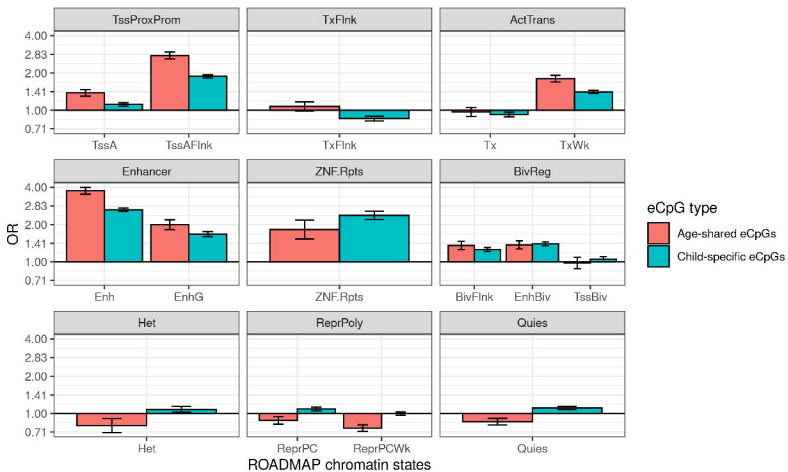


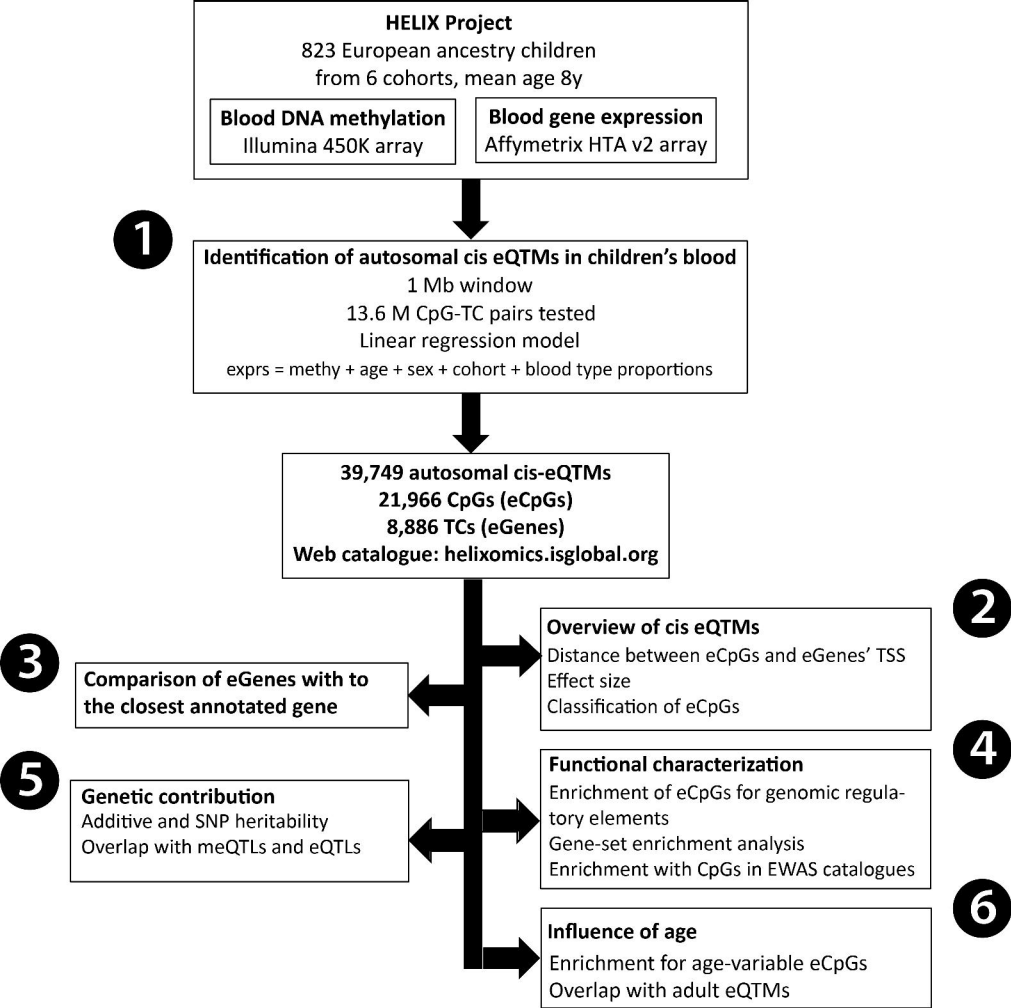




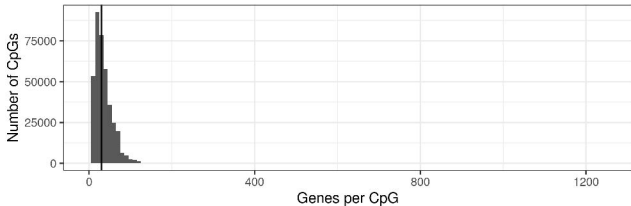








### CpGs pairing distribution



### Genes pairing distribution

