1 **Title:** Placental DNA methylation signatures of maternal smoking during pregnancy
2 and potential impacts on fetal growth
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48 **Competing interests**
49 The authors declare no competing interests.
50

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109   **Abstract**

110   Maternal smoking during pregnancy (MSDP) contributes to poor birth

111   outcomes, in part through disrupted placental functions, which may be reflected in the

112   placental epigenome. We meta-analyzed the associations between MSDP and placental

113   DNA methylation (DNAm) and between DNAm and birth outcomes within the

114   Pregnancy And Childhood Epigenetics (PACE) consortium (7 studies, N=1700, 344

115   with any MSDP). We identified 1224 CpGs that were associated with MSDP, of which

116   341 associated with birth outcomes and 141 associated with gene expression. Only 6 of

117   these CpGs were consistent with the findings from a prior meta-analysis of cord blood

118   DNAm, demonstrating substantial tissue-specific responses to MSDP. The placental

119   MSDP associated CpGs were enriched for growth-factor signaling, hormone activity,

120   inflammation, and vascularization, which play important roles in placental function. We

121   demonstrate links between placental DNAm, MSDP and poor birth outcomes, which

122   may better inform the mechanisms through which MSDP impacts placental function and

123   fetal growth.

124

125   **Keywords:** DNA methylation, epigenetics, EWAS, pregnancy, smoking, birth weight,

126   gestational age

127

128   Almost 1 in 10 pregnancies are impacted by the effects of maternal smoking during

129   pregnancy (MSDP), with state-specific prevalence ranging from as low as 1.8% to as

130   high as 27.1% in the USA[1], while in Europe, the prevalence of MSDP is estimated to

131   range between 4.2% and 18.9%[2]. Consequently, the numerous health effects of MSDP

132   remain a significant public health concern. The impact of this exposure on fetal

133   development has been the source of significant investigation, resulting in MSDP being

134   recognized as a cause of multiple negative pregnancy and birth outcomes[3].

135   The mechanisms that underlie this reproductive and developmental toxicity are

136   partially understood and include molecular and anatomical changes of the placenta[4,5].

137   Additionally, experimental mouse models have recently highlighted the critical roles of

138   proper placental function in ensuring successful pregnancy outcomes[6]. Epigenetic

139   responses to prenatal exposures have emerged as potential intermediate links between

140   early life exposures and developmental health outcomes, and epidemiologic studies of

141   DNAm, particularly multi-cohort collaborative efforts, are powerful approaches to

142   investigate these types of research questions[7]. So far, most studies of MSDP and

143   epigenetics have focused on DNA methylation (DNAm) variations in cord blood,

144   though some studies of placenta, peripheral blood and lung tissues have also been

145   performed[8]. The Pregnancy and Childhood Epigenetics (PACE) consortium[9] published

146   a large meta-analysis identifying thousands of MSDP-associated variations in DNAm

147   within cord blood and child peripheral blood[10]. However, the placental epigenome has

148   not been as thoroughly studied, though the placenta is likely a critical target organ of

149   MSDP-associated toxicity. A handful of prior studies have examined the relationships

150   between MSDP and (DNAm) in human placenta[11–14], identifying MSDP-associated loci

151   some of which have been suggested partially mediate the effects of MSDP on lower

152   birth weight[15].

153   These studies have begun to characterize the impact that MSDP has on the

154 human placental epigenome but have been limited by small sample sizes and have not

155 adjusted for placental tissue heterogeneity. We aimed to address these gaps by

156 performing a fixed effects meta-analysis examining the relationships between MSDP

157 and variations in the placental methylome across seven independent studies that are

158 members of the PACE consortium. We also aimed to gain insights into the potential

159 biological and health-related impacts of these associations by performing additional

160 analyses with nearby mRNA expression, as well as functional, regulatory, and

161 phenotypic enrichment, and a secondary meta-analysis of the associations between

162 placental DNAm and birth outcomes. This study represents the largest and most

163 comprehensive examination of MSDP associations with placental DNAm in human

164 populations and provides novel insights into the placental molecular responses to MSDP

165 and how these relate to fetal development.

166

167   **Results**

168   *Study population*

169   Seven American, Australian, and European studies (N=1,700) contributed to the

170   epigenome-wide association study (EWAS) linking MSDP to placental DNAm:

171   including Asking Questions about Alcohol in pregnancy (AQUA)[16], Study on the pre-

172   and early postnatal determinants of child health and development (EDEN)[17], Genetics of

173   Glucose regulation in Gestation and Growth (Gen3G)[18], Genetics, Early Life

174   Environmental Exposures and Infant Development in Andalusia (GENEIDA),

175   Environment and Childhood Project (INMA)[19], New Hampshire Birth Cohort Study

176   (NHBCS)[20] and Rhode Island Child Health Study (RICHS)[21]. For this meta-analysis,

177   344 (20.2%) mothers reported any MSDP, defined as any cigarette smoking during any

178   trimester of pregnancy. Any MSDP tended to be less prevalent in the cohorts from

179   Canada and the USA compared to those from Australia and Europe (Table 1). Three

180   cohorts (N=795, EDEN, GENEIDA and INMA) contributed to the EWAS of sustained

181   MSDP, defined as maternal smoking throughout pregnancy, among which 163 (20.5%)

182   mothers reported sustained MSDP. Distributions of covariates by cohort are provided in

183   the supplementary materials (Excel Table S1).

184   *Genome-wide DNAm meta-analyses*

185   We produced four statistical models for each CpG site, modeling the

186   associations between DNAm with both any and sustained MSDP, with and without

187   adjustment for putative cellular heterogeneity which was estimated with a reference free

188   deconvolution algorithm[22]. Models were adjusted for maternal age, parity, and maternal

189   education. Genomic inflation factors from the meta-analyses ranged from $\lambda=2.0$ to 2.8

190   (Excel Table S2, Supplemental Figure S1). Any MSDP was associated with DNAm at

191   532 CpG sites after Bonferroni adjustment (Excel Table S3), while sustained MSDP

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192 was associated with DNAm at 568 CpG sites (Excel Table S4). After adjusting for

193 putative cellular heterogeneity, any MSDP was associated with 878 CpGs (Excel Table

194 S5), while sustained MSDP was associated with 894 CpGs (Excel Table S6). Among

195 these, 10.7% of models for any MSDP and 3.9% of models for sustained MSDP, not

196 adjusted for cell type, yielded heterogeneity p-values < 0.01, while only 5.5% of the

197 models for any MSDP and 3.5% for sustained MSDP, adjusted for putative cellular

198 heterogeneity, produced heterogeneity p-values < 0.01. Thus, heterogeneity in the

199 associations across cohorts was lowest in the analyses that were adjusted for putative

200 cellular heterogeneity and the CpGs from these models that yielded Bonferroni-

201 significant associations (1224 unique CpGs) were carried forward for secondary

202 analyses.

203   Overall, the Bonferroni significant CpGs were distributed throughout the

204 genome, and the majority (68%) of CpGs exhibited lower DNAm in association with

205 any and sustained MSDP (Figure 1). Of the 548 CpGs that yielded Bonferroni

206 significant associations with both any and sustained MSDP, the absolute values of the

207 parameter estimates were greater for the models of sustained MSDP compared to the

208 estimates for any MSDP (Excel Table S7). In a secondary analysis that restricted the

209 any MSDP models to the same three cohorts that contributed to the sustained MSDP

210 models (EDEN, GENEIDA, and INMA), we again found that almost all CpGs (544 out

211 of 548) exhibited increased magnitudes of association with increased duration of

212 exposure (Excel Table S7, Supplemental Figure S2).

213   The most notable association was observed at cg27402634, located upstream of

214 the *LEKR1* gene and the non-coding RNA *LINC00886*, which showed the largest

215 differential DNAm and smallest p-values in all meta-analyses. Placentas that were

216 exposed to any MSDP had 22.95% lower DNAm (95% CI: 21.01-24.99% lower

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217   DNAm; p-value = 5.35E-119) and those exposed to sustained MSDP had 25.78% lower

218   DNAm (95% CI: 24.02-27.54% lower DNAm; p-value = 7.22E-181) when compared to

219   mothers that did not smoke at all during pregnancy. Though all cohorts observed

220   substantial hypomethylation with MSDP at this CpG, the actual estimates of the

221   associations were highly variable between cohorts for models of any MSDP

222   (heterogeneity p-value = 2.66E-15), but relatively consistent for models of sustained

223   MSDP (heterogeneity p-value = 1.55E-01) (Figure 2A). Overall, we consistency in the

224   associations across cohorts for the vast majority of the 1224 CpGs that yielded

225   Bonferroni-significant associations via meta-analysis: 92% and 97% of these CpGs

226   yielded heterogeneity p-values > 0.01, for their associations with any and sustained

227   MSDP respectively. In addition to cg27402634, we highlight those relationships that

228   yielded that largest magnitudes of association: ($|\beta_{\text{Any MSDP}}|$) > 0.05 for cg26843110

229   (*EDC3*), cg20340720 (*WBP1L*), and cg17823829 (*KDM5B*) (Figure 2B-2D). We

230   identified numerous other noteworthy relationships but due to the large number of

231   Bonferroni-significant associations from our meta-analyses, we highlight the

232   relationship among the 20 most statistically significant CpGs from the primary meta-

233   analysis of any MSDP going forward (Table 2).

234   ***Expression Quantitative trait methylation (eQTM) analyses***

235   　　　We tested whether the DNAm levels at MSDP-associated CpGs were associated

236   with the expression of nearby (± 250kb of the CpG) mRNA from 194 placental samples

237   in the RICHS cohort, and corrected for multiple testing with the less conservative false

238   discovery rate (FDR) due to the smaller sample size. We identified 170 associations

239   between DNAm and gene expression within a 5% FDR (Excel Table S8), including 141

240   unique CpGs and 140 unique mRNAs; 72.4% of the eQTMs exhibited inverse

241   associations, while inverse associations were most common and statistical significance

9

242 was strongest for CpGs that were closer to the transcription start site (Supplemental

243 Figure S3). Among the top 20 CpGs from our meta-analysis, eight were identified as

244 eQTMs (5% FDR) (Table 3). Higher placental DNAm at cg27402634, was associated

245 with lower expression (β = -2.32, and FDR < 5%) of *LEKR1*. Additionally, DNAm at

246 cg17823829 (annotated to *KDM5B*) was most strongly associated lower expression of

247 *PPFIA4* (FDR < 5%), and DNAm at cg26843110 (annotated to *EDC3*) was nominally

248 associated with higher expression of *CSK*, while cg20340720 (annotated to *WBP1L*)

249 was not associated with any nearby mRNA expression.

250 ***Functional and regulatory enrichment analyses***

251 Enrichment analyses were performed to gain insights into which biological

252 processes may be impacted by these MSDP-associated CpGs. We performed gene-set

253 enrichment analyses, which included 565 genes annotated to CpGs associated with any

254 MSDP and 581 genes for sustained MSDP. 143 and 25 pathways were significantly (q-

255 value < 0.05) enriched among CpGs associated with any MSDP (Excel Table S9) and

256 sustained MSDP (Excel Table S10), respectively. In both gene-sets, "signaling by nerve

257 growth factor (NGF)" was the most significant pathway (q-value = 2E-04 for any

258 MSDP and q-value = 9.6E-03 for sustained MSDP). Other significantly enriched

259 pathways were related to growth factors (VEGF, EGF, PDGF, IGF1R), hormones

260 (aldosterone, insulin, TSH, GnRH), interleukins (IL2, IL4, IL7), myometrial

261 contraction, vascular smooth muscle contraction, thrombin and platelet activation,

262 signaling and aggregation. We also tested whether the genes annotated to MSDP-

263 associated CpGs were enriched for regulatory regions of specific transcription factors

264 (TFs). Most notably, our MSDP-associated CpGs were enriched for genes regulated by

265 GATA1 and GATA2, as well as by RUNX1, TP63, SMAD4, AR, TP53 or PPARG

266 (Excel Tables S11-S12).

10

267 We then examined whether the MSDP-associated CpG sites were enriched for

268 CpG island locations, allele-specific germline differentially methylated regions

269 (gDMR)[23], regulatory features from the placenta specific 15-chromatin state annotation

270 from ROADMAP[24], or placenta specific partially methylated domains (PMD)[25], which

271 contain placenta-specific repressed genes (annotated to the results files in

272 Supplementary Tables S3-S6). The MSDP-associated CpGs were depleted in PMDs

273 (Supplemental Figure S4), highly enriched in placenta enhancers and depleted in

274 transcription start sites and inactive states (Supplemental Figure S5). Additionally,

275 MSDP-associated CpGs were depleted in CpG islands and shores, while enriched in

276 CpG island shelves and open sea positions (Supplemental Figure S6). We identified 3

277 CpGs (cg05211790 and cg16360861 at *RAI14*, and cg05575921 at *AHRR* gene) that

278 were within two candidate maternal gDMRs, but our overall set of MSDP-associated

279 CpGs were neither depleted nor enriched for confirmed gDMRs (Supplemental Figure

280 S7).

281 *Phenotype enrichment analyses*

282 The genes annotated to our MSDP-associated CpGs were tested for phenotype

283 enrichment using data from the database of Genotypes and Phenotypes (dbGAP). Our

284 MSDP-associated genes were enriched for numerous phenotypes in dbGAP, including

285 several adiposity phenotypes (body mass index (BMI), waist-hip ratio, and obesity),

286 blood pressure, cardiovascular diseases, type 2 diabetes, asthma and respiratory function

287 (Excel Tables S13-S14).

288 *Proximity to genetic variants for birth outcomes*

289 We aimed to understand whether the MSDP-associated CpGs that we identified

290 co-localize within the same genomic regions as genetic variants that have been

291 associated with birth outcomes via genome-wide association studies (GWAS). Thus we

292   investigated whether MSDP-associated CpGs were within ± 0.5 Mb (1 Mb window) of

293   single nucleotide polymorphisms (SNPs) that have been associated with birth weight

294   (BW), birth length (BL), head circumference (HC) and gestational age (GA)[26–31], which

295   have been added as annotations to the meta-analysis results files (Excel Tables S3-S6).

296   Of the 324 birth outcome SNPs in autosomal chromosomes, 94 SNPs (83 loci) and 108

297   (97 loci) were within 0.5 Mb of CpGs that were associated with any or sustained

298   MSDP, respectively (Excel Tables S15). Overall ~16% of the 1224 MSDP-associated

299   CpG sites were within 0.5 Mb of birth outcome SNPs, including cg27402634 (*LEKR1*),

300   cg26843110 (*EDC3*) and cg20340720 (*WBP1L*). We also explored whether our MSDP-

301   associated CpGs may be biased by methylation quantitative trait loci (mQTLs), in

302   which SNPs influence the methylation levels at nearby CpGs. Two studies have

303   examined this question in human placenta, identifying 866[32] and 4,342[33] placental

304   mQTLs. Our findings did not appear to be biased by genetic variation as only 9 of the

305   1,224 MSDP-associated CpGs are previously characterized placental mQTLs.

306   ***Association of DNAM at smoking associated loci and smoking related birth outcomes***

307   We also performed a second meta-analysis to examine the relationships between

308   DNAm with gestational age at birth, preterm birth, BW, BL, and HC z-scores, outcomes

309   that are known to be related to maternal smoking. Of the 1224 CpGs tested, 341

310   (27.9%) were related to at least one birth outcome after Bonferroni-adjustment

311   (0.05/1224). The majority of birth outcome associations were related to gestational age

312   at birth (298 CpGs) (Excel Table S16). Preterm delivery, for which only two cohorts

313   could contribute data (EDEN and NHBCS), produced similar associations found for

314   GA, though fewer CpGs were statistically significant (Excel Table S17). We also found

315   that numerous loci were associated birth size z-scores, with the majority of these being

316   associated with BW (43 CpGs) (Excel Table S18), followed by BL (20 CpGs) (Excel

317   Table S19) and HC (4 CpGs) (Excel Table S20). Some of the CpGs associated with GA

318   were also associated with birth size measurements, even though they were standardized

319   for GA, suggesting independent associations with both gestational duration and fetal

320   growth (Supplemental Figure S8A), including 6 CpGs (annotated to *SYNJ2*, *PXN*,

321   *PTPRE*, *IGF2BP2*, 4q21.1) shared between GA and BW, and 2 (annotated to *POLR3E*

322   and *LOC441869*) shared between GA and BL. Among the CpGs that yielded at least

323   one Bonferroni-significant association with birth outcomes, CpGs that tended to have

324   positive associations with birth outcomes clustered together and were typically

325   hypomethylated with MSDP, while CpGs that exhibited inverse associations with birth

326   outcomes tended to be hypermethylated with exposure to MSDP (Supplemental Figure

327   S8B).

328   Among our top 20 CpGs that were associated with any MSDP, 5 were associated

329   with GA at birth and 4 were associated with BW z-scores after Bonferroni adjustment

330   (Table 3). DNAm at cg27402634 (*LEKR1*) and cg20340720 (*WBP1L),* both located

331   close to BW-SNPs and for which MSDP associated with lower DNAm, were associated

332   with larger BW (p-value = 6.71E-07, and p-value = 2.42E-07). On the other hand,

333   DNAm at cg26843110 (*EDC3;* hypomethylated in response to MDSP and also close to

334   BW-SNPs) and at cg17823829 (*KDM5B;* hypermethylated) were associated with longer

335   and shorter gestational ages at birth, respectively (p-value = 5.09E-12, and p-value =

336   9.12E-06). Forest plots of BW z-scores and GA for these four CpGs are shown in

337   Figure 3.

338   We summarize the results for all secondary analyses with a circos plot, for those

339   548 CpGs that yielded Bonferroni significant associations with both any and sustained

340   MSDP (Figure 4). Among these, 21 CpGs were associated both with mRNA expression

341 (FDR < 5%) and at least one birth outcome (Bonferroni-significant), which have been

342 annotated with the gene symbols from their respective eQTM models.

343 ***Comparison with smoking-sensitive CpGs in cord blood***

344 We assessed whether the DNAm signatures of MSDP in the placenta were

345 consistent with MSDP associations in cord blood previously reported by the PACE

346 consortium[10]. Only nine CpGs annotated to seven unique genes (*AHRR, CYP1A1,*

347 *GNG12*, *PXN*, *RNF122*, *SLC23A2*, and *ZBTB4*) yielded Bonferroni-significant

348 associations in both tissues, out of 1224 CpGs from our study and 568 CpGs from the

349 cord blood study (Table 4). Of note, the CpGs within *CYP1A1* and *RNF122* showed

350 opposite directions of association with MSDP in cord blood and placenta. We also

351 compared the parameter estimates from our study that yielded associations with MSDP

352 within 5% FDR to the parameter estimates of those CpGs described in cord blood also

353 within a 5% FDR[10]. There was no overall correlation ($r^2<0.1$) of the regression

354 coefficients across these two tissues (Supplemental Figure S9).

355

Discussion

We identified 1224 CpG sites with placental methylation levels that were associated with any or sustained MSDP. Differential DNAm was greater with increased duration of exposure at all loci that were associated with both any and sustained MSDP, and a large proportion of the MSDP-associated CpGs were related to birth outcomes. Those CpGs that were observed to have higher DNAm associated with MSDP, tended to be inversely associated with gestational age and birth size, while CpGs exhibiting lower DNAm with MSDP tended to be positively associated with gestational age and birth size.

The MSDP-associated loci with the most statistically significant association (cg27402634), also identified in prior smoking EWAS of placental tissues[15], yielded dramatically lower DNAm levels in association with MSDP exposure. This effect-size is much larger in magnitude (~25% difference for sustained MSDP) compared to what has generally been observed in most exposure-focused EWAS, though within the same range as a CpG site in *AHRR* (cg05575921; 18% difference between exposed and unexposed) from a prior EWAS of current smoking and blood DNAm[34]. Additionally, decreased placental DNAm at cg27402634 correlates with increased expression of *LEKR1,* and associates with smaller BW and BL. Thus MSDP-associated hypomethylation at this CpG would be consistent with the well-known effect of maternal smoking resulting in shorter gestation and smaller birth size.

The functional activities of cg27402634, or corresponding *LEKR1* gene, in human placental tissues are not known. However, GWAS findings provide evidence that genetic variants within this region might be involved in fetal growth and possibly metabolic programming. For instance, the SNP rs1482852 or its proxies (rs900400; rs13322435) have been associated with different parameters of fetal growth[35], adiposity

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381   in newborns[36,37], maternal adiponectin levels, cord blood leptin[37], and insulin release

382   after an oral glucose challenge[38]. These findings from genetic studies in combination

383   with our current study, implicate that this locus on chromosome 3 (3q25.31) contains

384   very active determinants of growth regulation and metabolic activity, and that placental

385   DNAm at cg27402634 is highly responsive to maternal smoking. Future mechanistic

386   work is necessary to investigate whether the placental epigenetic regulation at this locus

387   specifically influences placental functions and/or overall growth and metabolic

388   functions of the developing fetus.

389   We identified numerous other notable MSDP-associated loci in addition to those

390   of cg27402634 (*LEKR1*), and highlight those CpGs yielding the strongest magnitudes of

391   effect (cg20340720, cg26843110, and cg17823829). MSDP was associated with lower

392   DNAm at cg20340720, located within *WBP1L* (also annotated as *C10orf26*), while

393   lower DNAm at this CpG correlated with lower with BW and BL z-scores. Genetic

394   variants nearby to this CpG have been related to BW[31] and blood pressure[39]. We also

395   observed lower DNAm with MSDP at cg26843110, which is within the body of the

396   *EDC3* gene, and is nearby to SNPs associated with BW (rs3784789[31]). Lower DNAm at

397   cg26843110 associated with shorter GA at birth, and decreased expression of *CSK*,

398   which is involved in trophoblast differentiation[40] as well as blood pressure and

399   aldosterone regulation[41]. Finally, cg17823829 (annotated to *KDM5B*) was

400   hypermethylated with MSDP. Higher DNAm at this CpG correlated with shorter GA at

401   birth and with lower expression of *PPFIA4* gene, which can be induced in response to

402   hypoxia[42].

403   Our enrichment analyses identified numerous pathways that are critical to

404   placental growth and development, such as vascularization, hormone signaling and

405   inflammatory cytokines. Multiple pathways involving vascular endothelial growth

16

406 factors (VEGF) and nerve growth factors (NGF) populated the top of our enrichment

407 lists. The VEGFs and their receptors are required for all steps of placental

408 vascularization[43], while nerve growth factor (NGF) modulates immune activity,

409 inflammation and angiogenesis in the placenta[44]. Thus, our findings may be related to

410 perturbed placental vascularization or angiogenic signaling. Altered placental

411 vasculature is the most common placental pathology identified in pregnancy

412 complications[43], and MSDP can result in placental vascular remodeling[5]. Our findings

413 may represent, in part, an epigenetic footprint of the placental vascular and angiogenic

414 response to MSDP. Our CpGs were also enriched for genes regulated by specific

415 transcription factors, most notably GATA1 and GATA2. Together with PPARG and

416 TP63, GATA factors are part of the core transcriptional regulatory circuit that guides

417 and maintains proper trophoblast differentiation[45,46]. Placentas lacking *PPARG* have

418 lethal defects in placental vascularisation[43], and angiogenic activity is reduced in

419 placentas lacking *GATA2*[47]. Additionally, our MSDP-associated CpGs were enriched in

420 placental enhancers, while depleted in transcription start sites, inactive states, PMDs[25]

421 and gDMRs[23], overall suggesting that the CpG sites that we identified are located in

422 active regulatory regions.

423 Genes that are annotated to the CpGs that we studied have been linked to human

424 health and disease traits via dbGAP, including a number of conditions that are part of

425 the metabolic syndrome (BMI, obesity, cholesterol, blood pressure), for which prior

426 links to MSDP have been reported[48,49]. This may indicate that the placental genes whose

427 regulation is impacted by MSDP, are involved in energy uptake and expenditure, lipid

428 and glucose metabolism, blood pressure regulation, and inflammation, which are some

429 of the key physiological processes that are disrupted in the pathogenesis of metabolic

430 syndrome[50]. The MSDP-associated CpGs were also enriched for genes linked to asthma

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431   and impaired respiratory function, which are known to be caused by MSDP[51], but it is

432   unclear if altered regulation of these genes in the placenta has consequences on the

433   development of the respiratory system. Furthermore, 128 of the 324 SNPs that have

434   previously been associated with birth size or gestational age at birth[26–31] were in similar

435   genomic proximity (within 0.5 Mb) to our CpGs, suggesting the MSDP-associated

436   differential methylation in the placenta occurs in regions of the genome that are heavily

437   involved in growth and development. Many of these SNPs have been shown to be

438   related to birth outcomes, glycemic traits, blood pressure, and height, while the largest

439   GWAS of BW to date concludes that the link between lower BW and later cardio-

440   metabolic traits are largely driven by shared genetic effects[31]. Future research is needed

441   to characterize this convergence of genetic effects on growth and placental epigenetic

442   responsiveness to MSDP within similar genomic proximities. It is possible that MSDP,

443   genetic variation, and placental DNAm in these regions yield additive or interactive

444   effects on birth outcomes; prior studies have addressed this question in blood[52,53].

445   Additionally, while our MSDP-associated CpGs are in similar genomic regions (1 Mb

446   windows) with birth outcome SNPs, very few of our CpGs (9 of 1224) and very few of

447   the birth outcome SNPs (3 of 324) are known placental mQTLs[32,33].

448   We compared our findings to those of a previous PACE meta-analysis of MSDP

449   and cord blood DNAm[10]. Two of the CpGs that we identified within the *AHRR* gene

450   (cg05575921 an eQTM for *AHRR* in placenta, and cg21161138), have been consistently

451   observed to be sensors of MSDP exposure in cord blood. Only four other CpG sites,

452   (annotated to *GNG12*, *PXN*, *ZBTB4*, and *SLC23A2*) were differentially methylated in

453   both placenta and cord blood with the same direction of association in both tissues.

454   Additionally, three CpG sites within *CYP1A1* and *RNF122* were identified in both meta-

455   analyses but with different directions of association in cord blood versus placenta.

18

456   Interestingly, we observed *CYP1A1* to be hypomethylated in placenta with exposure to

457   MSDP, which is consistent with studies of adipose, skin, and lung tissues[54], but this

458   CpG was hypermethylated in the cord blood meta-analysis[10]. Additionally, the most

459   statistically significant association with MSDP in placenta (cg27402634, *LEKR1*) was

460   not associated with MSDP in the cord blood meta-analysis. These observations, and the

461   lack of overall correlation in regression coefficients when comparing placental and cord

462   blood responses to MSDP, suggest that there are unique tissue-specific molecular

463   responses to this exposure.

464   The above findings should be interpreted within the context this study's

465   limitations. MSDP was self-reported and subject to misclassification, though differential

466   misclassification likely would have biased our findings towards the null. We modeled

467   two different definitions for MSDP and found that the models with sustained MSDP

468   produced larger magnitudes of association, as has been previously found in studies of

469   blood DNAm[55]. Although these findings suggest that increased duration of MSDP is

470   associated with greater differential DNAm, we did not assess dose-response patterns (ie.

471   number of cigarettes or cotinine concentrations), which should be the focus of future

472   investigations. Our study predominantly consisted of samples from mother-infant pairs

473   of European ancestry, and thus additional studies involving diverse racial and ethnic

474   backgrounds are needed in order to improve the generalizability of these findings.

475   While it is unlikely that placental DNAm would influence maternal smoking, the

476   observed associations between DNAm levels and reproductive outcomes could be due

477   to reverse causation. Placenta is a heterogeneous tissue with multiple different cell

478   types[56] that serve different functions and thus have different epigenetic states[57]. To

479   correct for this, we estimated and adjusted for variability in placental DNAm that may

480   be due to tissue heterogeneity. We utilized a data driven approach that was not based on

19

481   a methylome reference, as no references for placental cell-type methylomes are

482   currently available. Adjustments for these estimates of putative cellular admixtures did

483   reduce heterogeneity in the meta-analyses, thus improving the consistency of observed

484   associations between MSDP and DNAm across these independent cohorts. However, it

485   is possible that residual confounding may have influenced some of our results.

486   Despite these limitations, our study had numerous strengths, including a large

487   overall sample-size and seven independent studies to identify these relationships. We

488   used harmonized definitions of exposure variables and covariates, standardized

489   protocols for quality control and pre-processing of DNAm data, and standardized

490   methods for estimating/adjusting for tissue heterogeneity and for statistical analyses.

491   We also performed secondary analyses involving mRNA expression, functional and

492   phenotype enrichment, overlap with GWAS hits for reproductive outcomes, and meta-

493   analyses of DNAm variation with birth outcomes to provide biological and health-

494   related interpretations of our findings.

495   We identified a DNAm signature of MSDP in the placenta that shows substantial

496   differences from that observed in cord blood, most notably the CpG in close proximity

497   to *LEKR1*. Many of the identified MSDP-associated loci are involved in biological

498   process that are known to play critical roles in placental development, including

499   vascularization, angiogenesis, and inflammation. Additionally, a large proportion of the

500   MSDP-associated CpGs were also associated with GA at birth, or birth size z-scores,

501   suggesting these placental epigenetic variations may be intermediate molecular markers

502   between MSDP and these outcomes. Further study is required to determine whether

503   these epigenetic variations are causal mediators within these relationships or reflecting

504   other processes.

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507 **Material and methods**

508 *Participating cohorts*

509   Cohorts that are members of the PACE consortium were identified for

510 participation in the current study if they had existing DNAm data quantified from

511 placental tissue via the Illumina Infinium HumanMethylation450 BeadChip and if they

512 had obtained information on self-reported smoking during pregnancy. The seven

513 cohorts that contributed to the meta-analysis of any MSDP included AQUA[16], EDEN[17],

514 Gen3G[18], GENEIDA, INMA[19], NHBCS[20] and RICHS[21]. EDEN, GENEIDA and INMA

515 also contributed to the sustained MSDP stratified analyses. RICHS contributed RNAseq

516 data for analyses with mRNA expression. All cohorts acquired ethics approval and

517 informed consent from participants prior to data collection through local ethics

518 committees. Exclusion criteria for this study were: non-singleton births, pre-eclampsia

519 and DNAm not assessed in the fetal side of the placenta. All participants in the study

520 were of European ancestry, except 1.85% of EDEN mothers. Detailed methods for each

521 cohort are provided in the Supplementary Material (Supplemental Methods File).

522 *Tobacco smoking definitions*

523   Any MSDP was defined as "yes" if mothers reported smoking cigarettes at any

524 time during pregnancy. Sustained MSDP was defined as "yes" when mothers reported

525 smoking cigarettes at least in the 1st and 3rd trimester of pregnancy. For both exposure

526 variables, the comparison group was defined as the mothers that reported no smoking

527 during any of the pregnancy.

528 *Placental genome-wide DNAm data acquisition, quality control and normalization*

529   Placental DNAm from the fetal side was assessed with the Infinium Human-

530 Methylation450 array (Illumina, San Diego, CA USA). See Supplementary Methods file

531 for extra details on placenta collection, DNA extraction and DNAm acquisition in each

22

532 cohort. Quality control of DNAm was standardized across all cohorts. Low quality

533 samples were filtered out and probes with detection p-values > 0.01 were excluded.

534 Beta-values were normalized via functional normalization[58] and beta-mixture quantile

535 normalization (BMIQ)[59] was applied to correct for the probe type bias. Cohorts applied

536 ComBat to remove batch effects when applicable. Probes that hybridize to the X/Y

537 chromosomes, cross-hybridizing probes and probes with SNPs at the CpG site,

538 extension site, or within 10 bp of the extension site with an average minor allele

539 frequency > 0.01   were filtered out[60]. Overall, 418,639 probes and 415,396 were

540 available for modelling any MSDP and sustained MSDP, respectively. Finally, DNAm

541 extreme outliers (<25th percentile – 3*IQR or >75th percentile + 3*IQR across all the

542 samples) were trimmed.

543 ***Estimates of putative cellular heterogeneity***

544   Placental putative cellular heterogeneity was estimated from DNAm data using a

545 reference-free cell-mixture decomposition method[61]. The number of surrogate variables

546 ranged from 2 to 5 depending on the cohort. Models for differential DNAm were

547 corrected for the number of surrogate variables minus one to reduce multi-collinearity.

548 ***Genome-wide differential DNAM analyses***

549   Within each cohort, robust linear regression from the "MASS" package[62] in R

550 were used to account for potential heteroskedasticity while testing the associations

551 between normalized DNAm beta values at each CpG with any MSDP and sustained

552 MSDP. Models were adjusted for maternal age, parity, maternal education and cohort-

553 specific variables first unadjusted for putative cellular heterogeneity then adjusted for

554 cellular heterogeneity. We performed inverse variance-weighted fixed-effects meta-

555 analyses using METAL[63]. The meta-analysis was performed independently by two

556 groups to ensure consistent results. CpGs not retained in at least 2 cohorts were filtered

23

557   out. We used the Bonferroni adjustment to control for multiple testing. To examine

558   whether increased duration of exposure (sustained smoking versus any smoking)

559   yielded increased magnitudes of association, we calculated the percent change in the

560   coefficients between the two models $(|\beta_{Sustained}| - |\beta_{Any}|)/|\beta_{Any}| * 100$. Secondary analyses

561   were only performed on CpGs that yielded Bonferroni significant associations with any

562   or sustained MSDP in models that were adjusted for putative cellular heterogeneity.

Expression quantitative trait methylation (eQTM) loci

564   We performed expression quantitative trait methylation (eQTM)[64] analyses in

565   the RICHS cohort. Transcription was measured via RNA-seq on 194 placentas. The

566   details of sample collection, assay, and QC for the RNA-seq data are presented in detail

567   elsewhere[65], and summarized in the Supplementary Material (Supplementary Methods

568   File). In this dataset, we identified 6523 unique transcripts annotated to an Ensembl ID

569   (GrCh37/hg19) and with a transcriptional start site (TSS) within 250 kb upstream or

570   downstream of 1184 out of the 1224 candidate CpGs. The association between DNAm

571   and expression levels was assessed via 10295 linear regression models using the MEAL

572   package[66] in R. We report the results for all models yielding nominally significant

573   associations (raw p-values <0.05), statistically significant eQTMs were determined at

574   5% FDR.

CpG site annotation

576   We annotated CpGs to genes and CpG islands with notations from the Illumina

577   HumanMethylation 450K annotation file, and with several regulatory features using

578   publicly available data: placental 15-chromatin states[67] released from the ROADMAP

579   Epigenomics Mapping Consortium[24] (ChromHMM v1.10), placental partially

580   methylated domains (PMDs)[25] and placental germline differentially methylated regions

581   (gDRMs)[23].

582   *Enrichment analyses*

583   Functional enrichment analyses were performed at the gene level via

584   ConsensusPathDB[68] using KEGG, Reactome, Wikipathways, Biocarta as reference

585   gene-sets. ConsensusPathDB performs a hypergeometric test and corrects multiple-

586   testing with FDR. Enrichment for transcription factors and for phenotypes were

587   assessed at the gene level with EnrichR using ENCODE and ChEA consensus TFs from

588   ChIP-X database, and dbGaP database, respectively. EnrichR results were ranked using

589   the combined score (P-value computed using Fisher exact test combined with the z-

590   score of the deviation from the expected rank).[69] Enrichment for regulatory features was

591   assessed with the hypergeometric test, and P-values were Bonferroni-corrected for 15

592   (placental chromatin 15-states) and 6 (relation to CpG island) tests, respectively.

593   *Overlap of MSDP-sensitive CpG sites and birth outcome SNPs*

594   Co-localization between MSDP-associated CpGs in placenta with previously

595   identified BW, BL, HC and GA SNPs from the largest genome-wide association studies

596   (GWAS) to date[26–31] was assessed using the GenomicRanges package in R[70]. We

597   identified which CpGs were located within 1 Mb windows ($\pm$ 0.5 Mb) surrounding each

598   of the 324 autosomal SNPs, which correspond to 280 potential unique loci. Unique loci

599   were defined based on the criteria in Warrington et al. 2019[31], and linkage

600   disequilibrium in Europeans ($r^2 > 0.1$ in < 2Mb).

601   *Association between DNAm and birth outcomes*

602   Within each cohort, robust linear regression models were utilized to test the

603   association between normalized DNAm beta values at each CpG as the independent

604   variable and gestational age at birth (inverse normal transformation of sex residuals),

605   BW z-scores, BL z-scores, and HC z-scores as the dependent variables. Logistic

606   regression was used to examine the relationships between DNAm and pre-term birth

607   (defined as <37 weeks of gestation). Birth size z-scores were calculated using

608   international references from the INTERGROWTH-21st Project[71] and standardized by

609   both gestational age and newborn sex. Models were adjusted for maternal age, parity,

610   maternal education, cohort-specific variables (see Supplemental Methods) and putative

611   cellular heterogeneity. Inverse variance-weighted fixed-effects meta-analyses[63] were

612   again used to estimate pooled associations. Multiple testing was controlled with the

613   Bonferroni adjustment ($\alpha = 0.05/1224$).

614   ***Comparison of MSDP-sensitive CpGs in placenta with cord blood***

615   We examined the consistency between MSDP-sensitive CpGs in placenta and in

616   cord blood[10]. First we compared the coefficients from the models for sustained MSDP

617   in cord blood, unadjusted for cellular heterogeneity, to results for both any and

618   sustained MSDP in placenta, adjusted for cellular heterogeneity, using Pearson

619   correlation coefficients.

620   All DNAm data processing and analyses were conducted in R, with the

621   exception of the meta-analyses which were performed with METAL.

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811

812 **Author contributions**

813 TME, MVU, CJM, JL, MFH and MB conceived of and designed the study. Study-

814 specific analyses were completed by JMC, (AQUA), ES (EDEN), AC (Gen3G), PCS

815 and JMM (GENEIDA), MVU (INMA) and TME (NHBCS and RICHS). MV meta-

816 analysed these results. TME, MV and MB performed the follow-up analyses. TME,

817 MVU, JS, CJM, MFH, and MB interpreted the results. TME, MVU, and MB wrote the

818 first draft of the manuscript. All authors (TME, MVU, ES, AC, ML, JMC, CL, ERB,

819 NFJ, BH, PP, BGA, JH, MAD, MRK, CI, LB, PCS, YJL, KH, TB, MAC, JMM, EM,

820 JC, MFF, JT, AGM, SJL, JS, CJM, JL, MFH, MB) read and critically revised drafts of

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26   Table 1: Distribution of any and sustained MSDP within participating cohorts.

Cohort	Country	Any MSDP			Sustained MSDP		
		Total N	N (%) Non-smokers	N (%) Smokers	Total N	N (%) Non-smokers	N (%) Smokers
AQUA	Australia	99	75 (75.76%)	24 (24.24%)	-	-	-
EDEN	France	647	446 (68.93%)	201 (31.07%)	570	446 (68.93%)	124 (21.75%)
Gen3G	Canada	151	138 (91.39%)	13 (8.61%)	-	-	-
GENEIDA	Spain	87	67 (77.01%)	20 (22.99%)	82	67 (77.01%)	15 (18.29%)
INMA	Spain	166	119 (71.69%)	47 (28.31%)	143	119 (71.69%)	24 (16.78%)
NHBCS	USA	310	290 (93.55%)	20 (6.45%)	-	-	-
RICHS	USA	240	221 (92.08%)	19 (7.92%)	-	-	-
TOTAL		1700	1356 (79.76%)	344 (20.24%)	795	632 (79.50%)	163 (20.50%)

27   MSDP = Maternal smoking during pregnancy.

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Table 2. Meta-analysis results from models of any and sustained MSDP, for the 20 CpGs with the smallest p-values for the model of any MSDP, and the percent increase in effect size between sustained and any MSDP (% Change); all models adjusted for maternal age, parity, education and putative cellular heterogeneity; CpGs that were not annotated with a gene name in the Illumina 450K annotation file have been annotated with their genomic region (ie. 4q12).

CpG Annotations			Any MSDP			Sustained MSDP			% Change
CpG ID	Location	Annotated Gene (Region)	β_1	S.E.	P-value	β_1	S.E.	P-value	
cg17823829	chr1:202765754	*KDM5B* (Body)	0.06	0.006	1.79E-22	0.086	0.008	3.80E-27	42.98
cg26045080	chr1:36807363	*STK40* (Body)	0.024	0.003	6.66E-19	0.032	0.003	4.86E-21	31.12
cg00534380	chr2:101766586	*TBC1D8* (Body)	-0.044	0.004	2.53E-25	-0.061	0.006	1.03E-28	38.46
cg19246018	chr2:240031588	*HDAC4* (Body)	0.015	0.002	1.39E-20	0.016	0.002	6.52E-20	8.28
cg23752985	chr2:85803571	*VAMP8* (TSS1500)	-0.019	0.002	1.31E-19	-0.025	0.003	3.77E-16	32.98
cg00666842	chr2:88366145	*SMYD1* (TSS1500)	0.033	0.003	1.17E-22	0.049	0.004	2.23E-34	48.17
cg27402634	chr3:156536860	3q25.31	-0.23	0.01	5.40E-119	-0.258	0.009	7.20E-181	12.33
cg09491670	chr4:53529646	4q12	0.016	0.002	1.60E-19	0.023	0.002	3.96E-29	43.31
cg25585967	chr5:14452105	*TRIO* (Body)	0.04	0.005	7.53E-19	0.061	0.006	2.16E-23	51.74
cg12291408	chr7:100037572	7q22.1	-0.036	0.004	1.43E-18	-0.052	0.005	2.34E-22	45.15
cg14214914	chr9:131870304	*CRAT* (Body)	0.036	0.004	1.57E-20	0.05	0.005	1.20E-26	41.29
cg20340720	chr10:104512523	*WBP1L* (Body)	-0.054	0.004	5.38E-42	-0.076	0.005	2.19E-58	40.52
cg26648103	chr11:66791718	*SYT12* (5'UTR)	-0.033	0.004	8.98E-21	-0.043	0.005	4.30E-20	30.51
cg26115089	chr11:93846406	*HEPHL1* (3'UTR)	0.025	0.002	1.33E-28	0.032	0.003	2.00E-27	24.8
cg26843110	chr15:74935742	*EDC3* (Body)	-0.056	0.005	1.74E-29	-0.082	0.007	1.74E-36	47.84
cg26433445	chr16:81764289	16q23.3	0.024	0.003	5.85E-20	0.034	0.003	1.34E-23	40.25
cg24177452	chr17:27494295	*MYO18A* (5'UTR)	0.025	0.003	3.40E-18	0.031	0.004	2.84E-18	27.35
cg06716730	chr17:35851459	*DUSP14* (5'UTR)	-0.022	0.003	1.40E-18	-0.032	0.003	4.47E-22	46.79
cg03313447	chr19:41829042	*CCDC97* (3'UTR)	-0.02	0.002	1.26E-22	-0.029	0.002	1.93E-34	45.73
cg02341503	chr20:45947123	*ZMYND8* (Body)	-0.02	0.002	1.81E-18	-0.025	0.003	4.83E-18	25.74

β_1 = Coefficient of the association between DNAm and MSDP; MSDP = Maternal smoking during pregnancy; S.E. = Standard Error.

Table 3. Results from eQTM models, DNAm versus gestational age at birth, and DNAm versus BW Z-score models that yielded at least nominally significant associations, among the 20 CpGs that yielded the most statistically significant associations with any MSDP in the primary meta-analysis (sorted in the same order as Table 2).

CpG ID	Annotated Gene	eQTM				Gestational Duration			BW Z-score		
		eQTM Gene	β_1	S.E.	P-value	β_1	S.E.	P-value	β_1	S.E.	P-value
cg17823829	KDM5B	PPFIA4	-2.53	0.7	4.05E-04	-1.18	0.27	9.12E-06	-1.02	0.26	9.72E-05
cg26045080	STK40	SH3D21	-4.26	0.77	8.34E-08	-1.41	0.49	4.19E-03	-	-	-
cg00534380	TBC1D8	TBC1D8	-2.29	0.51	1.13E-05	3.53	0.39	9.26E-20	1.48	0.39	1.62E-04
cg19246018	HDAC4	-	-	-	-	-1.39	0.59	1.74E-02	-	-	-
cg23752985	VAMP8	CAPG	6.33	1.75	3.96E-04	5.47	0.69	1.40E-15	-	-	-
cg00666842	SMYD1	-	-	-	-	-	-	-	-	-	-
cg27402634	3q25.31	LEKR1	-2.32	0.67	7.14E-04	-	-	-	0.92	0.19	6.71E-07
cg09491670	4q12	USP46	-3.59	-	4.91E-04	-	-	-	-	-	-
cg25585967	TRIO	-	-	-	-	-0.97	0.37	8.64E-03	-1.35	0.36	2.06E-04
cg12291408	7q22.1	ACTL6B	-4.89	-	1.12E-02	-	-	-	1.31	0.39	6.82E-04
cg14214914	CRAT	CRAT	-4.76	0.59	6.03E-14	-0.96	0.39	1.35E-02	-	-	-
cg20340720	WBP1L	-	-	-	-	-	-	-	1.86	0.36	2.42E-07
cg26648103	SYT12	-	-	-	-	1.06	0.43	1.29E-02	1.66	0.42	7.56E-05
cg26115089	HEPHL1	-	-	-	-	-1.1	0.55	4.43E-02	-1.48	0.55	6.71E-03
cg26843110	EDC3	CSK	0.57	0.25	2.18E-02	2.3	0.33	5.09E-12	1.28	0.33	1.19E-04
cg26433445	16q23.3	-	-	-	-	-	-	-	-2.99	0.61	9.06E-07
cg24177452	MYO18A	MYO18	-1.37	0.43	1.79E-03	-	-	-	-	-	-
cg06716730	DUSP14	DUSP14	-3.07	0.96	1.59E-03	3.72	0.54	7.87E-12	-	-	-
cg03313447	CCDC97	TGFB1	-10.88	1.41	7.27E-13	-2.48	0.74	7.79E-04	2.29	0.73	1.58E-03
cg02341503	ZMYND8	ZMYND8	-1.72	0.64	7.37E-03	1.44	0.63	2.29E-02	3.19	0.62	2.70E-07

β_1 = Coefficient of the association between DNAm and the outcome variable (mRNA expression, Gestational duration, or BW Z-score); BW = Birth weight; S.E. = Standard Error.

Table 4: Differentially methylated CpGs with MSDP in placenta (from our meta-analysis) and cord blood (from a published PACE meta-analysis[10]) that yielded Bonferroni-significant associations in both tissues.

CpG Annotation		Cord blood[*] (Sustained MSDP)			Placenta (Sustained MSDP)			Placenta (Any MSDP)		
CpG ID	Gene	β_1	S.E.	P-value	β_1	S.E.	P-value	β_1	S.E.	P-value
cg25189904	*GNG12* (TSS1500)	-0.024	0.002	1.38E-32	-0.020	0.003	2.54E-13	-0.014	0.002	1.99E-10
cg05575921	*AHRR* (Body)	-0.064	0.002	1.64E-193	-0.030	0.005	5.01E-09	-0.015	0.004	1.83E-04
cg21161138	*AHRR* (Body)	-0.022	0.001	1.78E-54	-0.026	0.004	5.25E-11	-0.018	0.003	2.55E-10
cg08327744	*RNF122* (Body)	-0.010	0.002	1.37E-08	0.020	0.003	3.12E-09	0.016	0.003	2.21E-09
cg15893360	*PXN* (Body)	-0.011	0.002	1.66E-08	-0.024	0.004	3.61E-08	-0.015	0.003	3.43E-06
cg12101586	*CYP1A1* (TSS1500)	0.045	0.003	3.67E-50	-0.045	0.008	7.15E-08	-0.026	0.006	9.12E-06
cg23680900	*CYP1A1* (TSS200)	0.003	0.001	8.73E-08	-0.018	0.002	6.26E-21	-0.006	0.001	7.35E-06
cg07565956	*ZBTB4* (5'UTR)	-0.006	0.001	2.50E-10	-0.023	0.003	4.02E-18	-0.016	0.002	2.26E-16
cg16547579	*SLC23A2* (5'UTR)	-0.013	0.002	2.00E-10	-0.048	0.009	1.47E-08	-0.031	0.007	1.42E-06

β_1 = Coefficient of the association between DNAm and MSDP; MSDP = Maternal smoking during pregnancy; S.E. = Standard Error

[*] Main model in Joubert et al: sustained MSDP adjusted for cell type heterogeneity
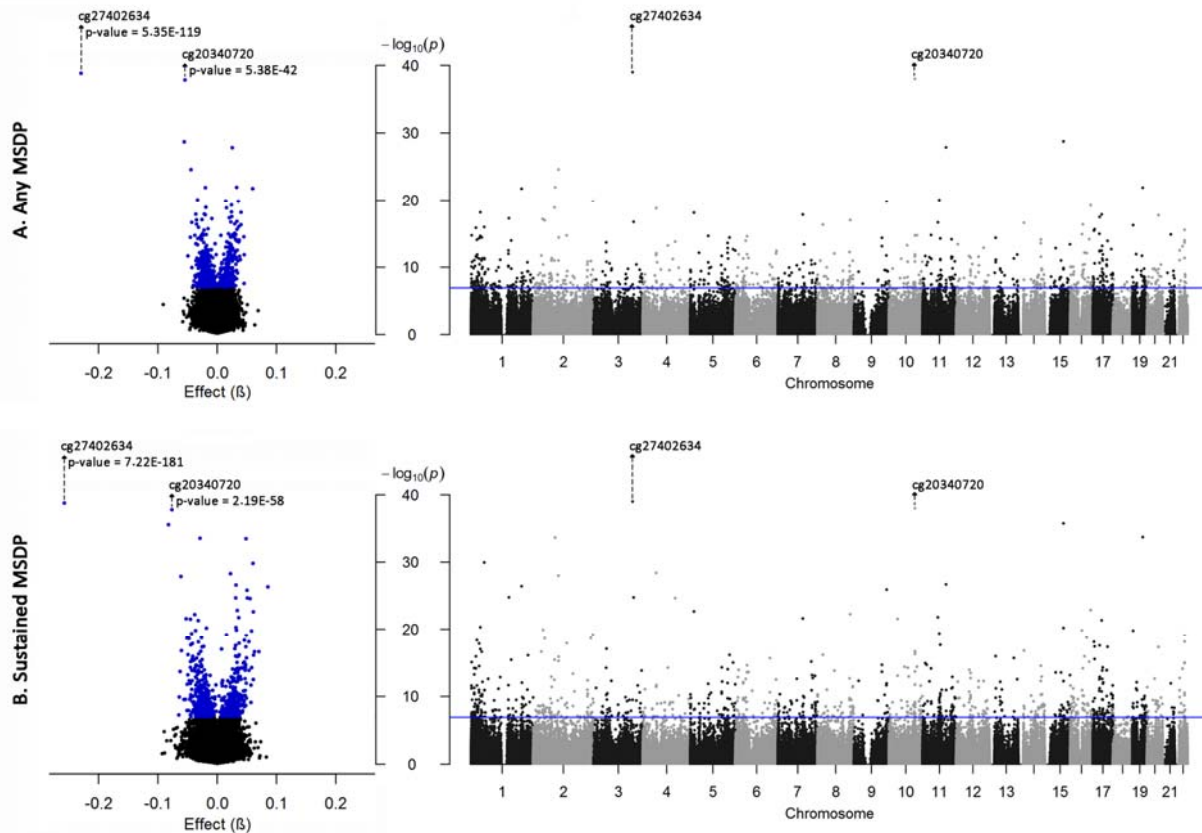
Figure 1. Volcano and Manhattan plots of the association between any MSDP (total N=1700, exposed = 344) (A) and sustained MSDP (total N=795, exposed = 163) (B) with placental DNAm adjusted for maternal age, parity, maternal education and putative cellular heterogeneity. In the volcano plots, the x-axis shows the difference in DNAm (effect) with a possible range between 0 and 1, while the x-axis in the Manhattan plot represents genomic location; both plots share the same y-axis with $-\log_{10}(P)$. Bonferroni thresholds for statistical significance are shown as blue dots and a blue horizontal line, for volcano and Manhattan plots, respectively. The y-axes were truncated to a minimum p-value of $1*10^{-40}$ (or maximum $-\log_{10}(P)$ of 40), to allow for better visualization of the majority of our results. The CpGs that were impacted by y-axis truncation are indicated with arrows and annotated with their actual p-values.
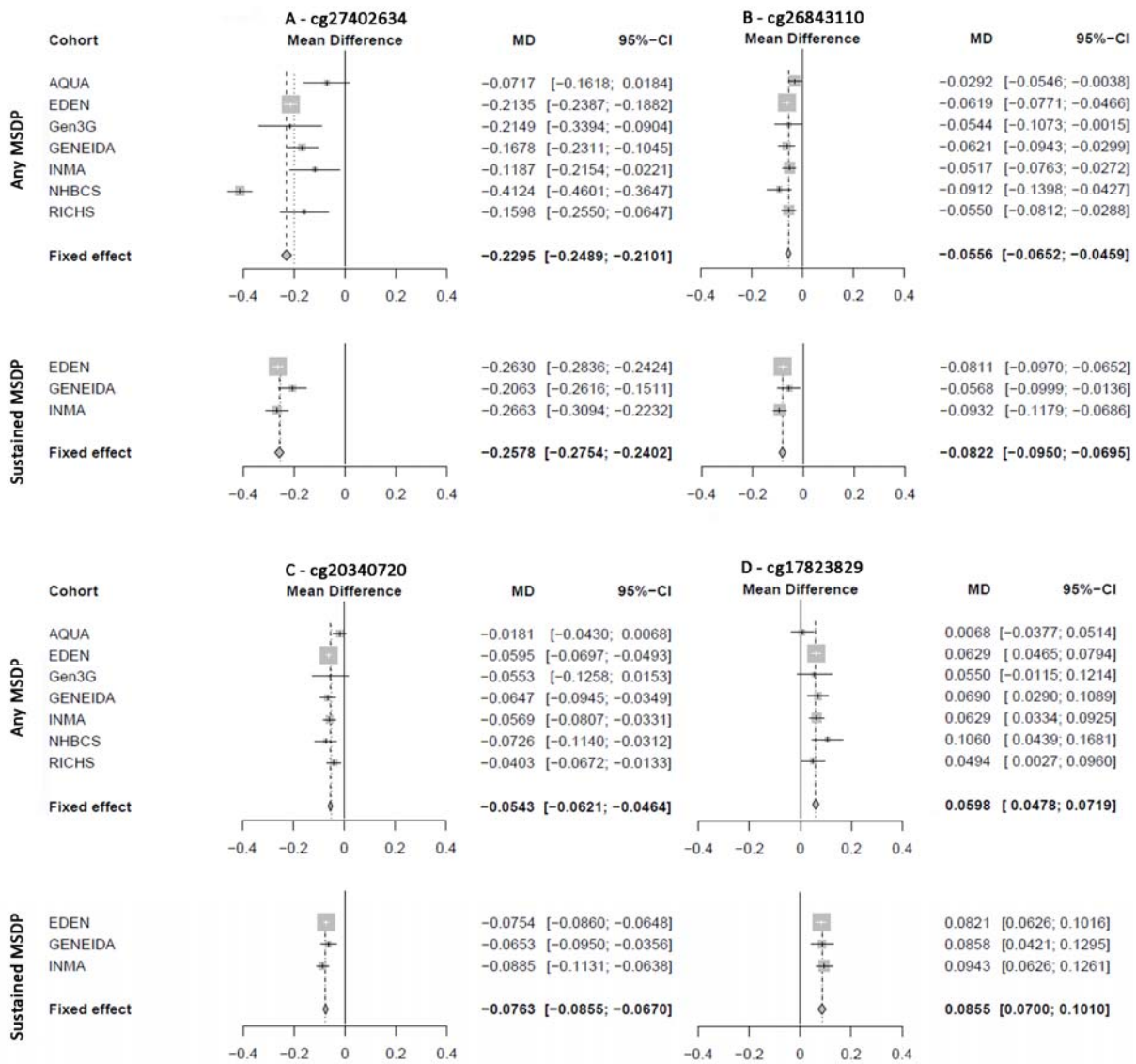
Figure 2. Forest plots of the cohort specific estimates of association and fixed-effect meta-analysis estimates of association between placental DNAm levels at (A) cg27402634, (B) cg26843110, (C) cg20340720, and (D) cg17823829 with any MSDP and sustained MSDP; models adjusted for maternal age, parity, maternal education, and putative cellular heterogeneity.
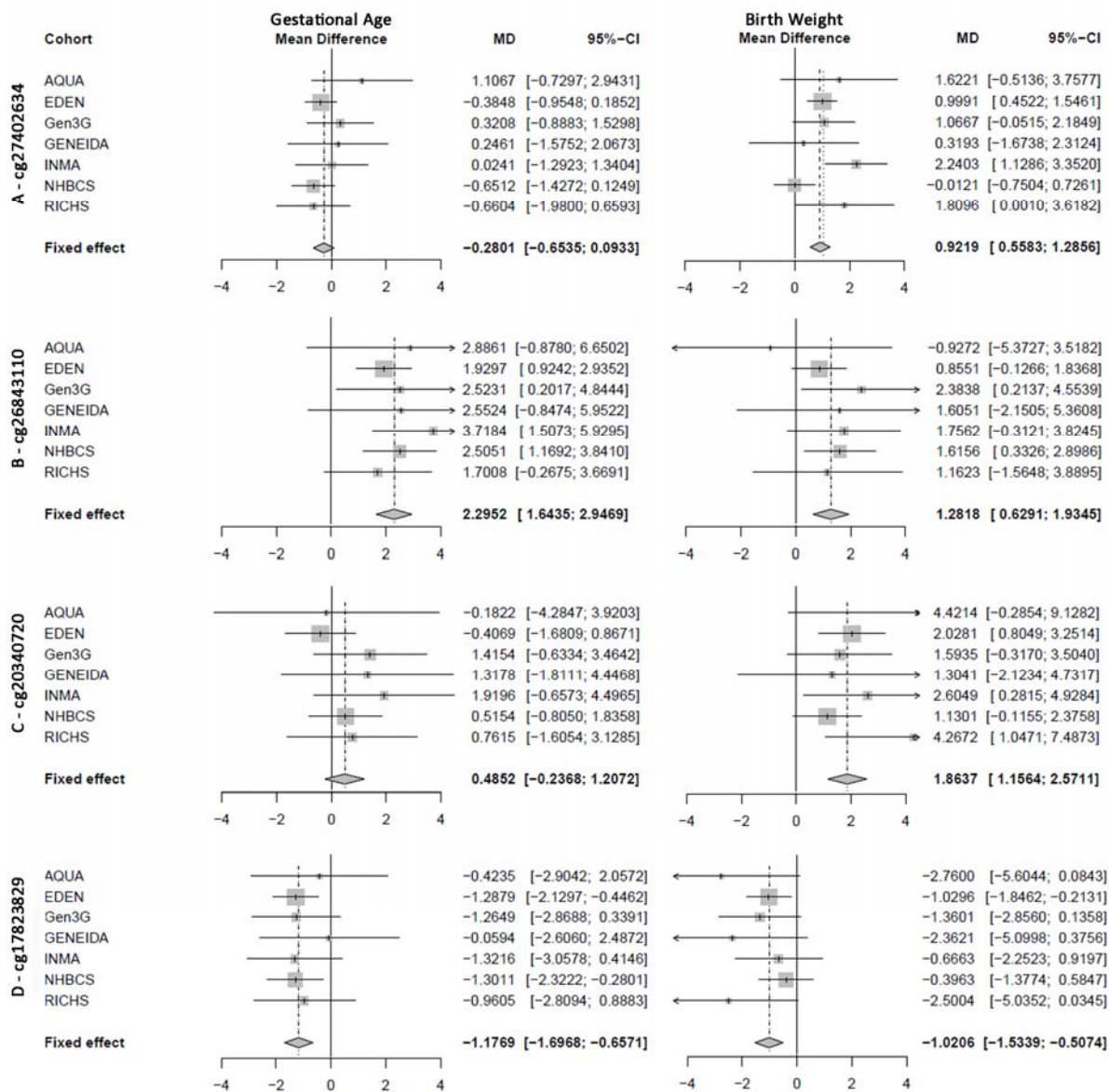
Figure 3. Forest plots of the cohort specific estimates of association and fixed-effect meta-analysis estimates of association between higher levels of placental DNAm at (A) cg27402634, (B) cg26843110, (C) cg20340720, and (D) cg17823829 with gestational age and birth weight z-scores; models adjusted for maternal age, parity, maternal education, and putative cellular heterogeneity.
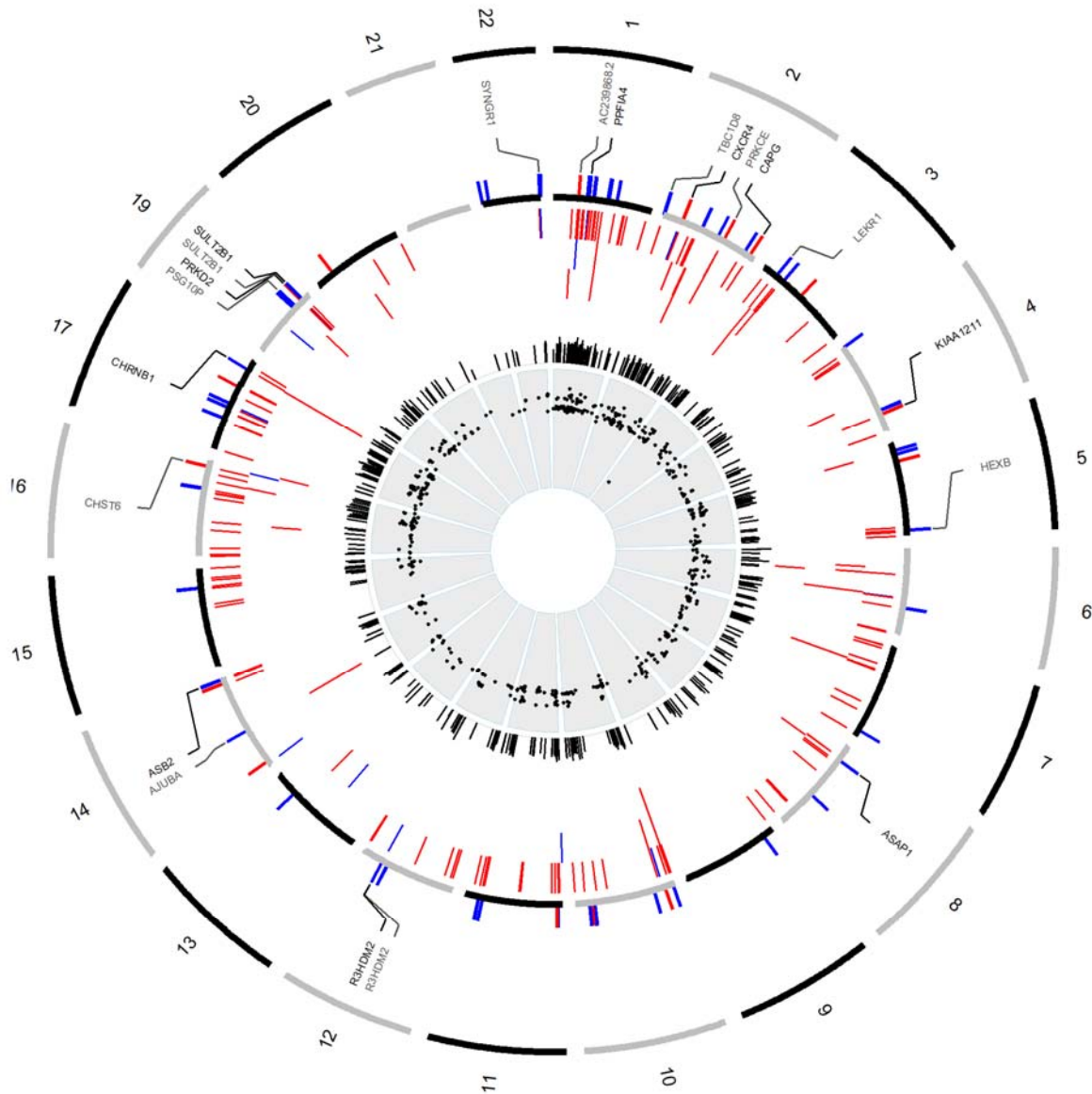
Figure 4. Circos plot summarizing all analyses for the 548 CpG sites that yielded Bonferroni-significant associations with both any and sustained MSDP. From inner to outer-band: Black points = differential DNAm between any- and no MSDP; Black Bars = percent increase in magnitude of association when comparing results from any MSDP to sustained MSDP; Color Bars = Positive (red) or Inverse (blue) associations between DNAm and HC, BL, BW, and GA, that yielded Bonferroni-significant associations; Color Bars = Positive (red) or Inverse (blue) associations between DNAm and nearby gene expression, that yielded an association at a 5% FDR; Names of eQTM genes were annotated to the outer band for CpGs that yielded an association between DNAm and expression with a 5% FDR and yielded an association between DNAm and at least one birth outcome after Bonferroni-adjustment.