

Title: Updates to data versions and analytic methods influence the reproducibility of results from epigenome-wide association studies

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1 **ABSTRACT**

2 **Introduction:** Biomedical research has grown increasingly cooperative, with several large consortia
3 compiling and sharing epigenomic data. Since data are typically preprocessed by consortia prior to
4 distribution, the implementation of new pipelines can lead to different versions of the same dataset.
5 Analytic frameworks also constantly evolve to incorporate cutting-edge methods and shifting best
6 practices. However, it remains unknown how differences in data and analytic versions alter the results
7 of epigenome-wide analyses, which has broad implications for the replicability of epigenetic
8 associations. Thus, we assessed the impact of these changes using a subsample of the Avon
9 Longitudinal Study of Parents and Children (ALSPAC) cohort.

10 **Methods:** We analyzed two versions of DNA methylation data, processed using separate preprocessing
11 and analytic pipelines, to examine associations between childhood adversity and prenatal smoking
12 exposure on DNA methylation at age 7. We performed two sets of analyses: (1) epigenome-wide
13 association studies (EWAS); (2) Structured Life Course Modeling Approach (SLCMA), a two-stage
14 method that models time-dependent effects. We also compared results from the SLCMA using more
15 recent methodological recommendations.

16 **Results:** Differences between ALSPAC data versions impacted both EWAS and SLCMA analyses,
17 yielding different sets of associations at conventional p-value thresholds. However, the magnitude and
18 direction of associations was generally consistent between data versions, regardless of significance
19 thresholds. Updating the SLCMA analytic version similarly altered top associations, but time-
20 dependent effects remained concordant.

21 **Conclusions:** Changes to data and analytic versions influenced the results of epigenome-wide studies,
22 particularly when using p-value thresholds as reference points for successful replication and stability.

23 **Keywords:** ALSPAC, epigenetic data versions, analytic versions, updates/revised, adversity, DNA
24 methylation, reproducibility.

25 INTRODUCTION

26 Biomedical science has become increasingly cooperative over the past decade. The emergence of large
27 datasets, combined with the small effects of biological measures on complex traits, has fueled such
28 cooperation, making global collaboration with researchers more important now than ever. Access to
29 large-scale data has emphasized the importance of identifying both replicable and stable findings, both
30 across and within research studies. As such, large consortia, including birth cohorts, have become an
31 integral part of these collaborative efforts, generating and compiling large amounts of research data
32 ranging from behavioral and clinical markers to molecular and genetic measures. These data are often
33 made available to collaborators and other researchers worldwide, facilitating the interrogation of
34 broader research questions and enabling replication efforts.

35 Epigenetic data are one key data type collected within these consortia. Epigenetics refer to mechanisms
36 that can result in heritable changes to gene expression without altering genetic sequences ¹. DNA
37 methylation (DNAm) is the most common type of epigenetic mechanism measured in human studies.
38 DNAm occurs when a methyl residue is added to cytosine residues, typically in the context of cytosine-
39 guanine dinucleotides (CpG). DNAm is both stable over time and responsive to external signals in
40 certain genomic contexts, which highlights its potential as a biomarker and mechanism for the
41 biological embedding of environmental factors ². As such, epigenome-wide association studies
42 (EWAS) have exploded in popularity, with over 1,600 papers on EWAS published since 2015.

43 To facilitate the sharing of DNAm data, datasets are often processed by the individual cohorts prior to
44 distribution. However, due to both technological and conceptual developments over time, the data
45 available from large cohorts will sometimes become outdated, requiring the distribution of revised
46 versions to collaborators. In addition, individuals in longitudinal studies occasionally withdraw consent
47 to share their data, reducing the overlap of samples between different data versions. At the same time,
48 analytic frameworks are constantly updated and improved upon, resulting in newer cutting-edge

49 methods and shifting analytic best practices ³. Yet, the extent to which differences in data versions and
50 analytic pipelines lead to meaningful differences in analytic results remains unclear. This raises an
51 important question as to the replicability and stability of findings across and within studies, which may
52 influence our interpretation of epigenome-wide associations in biomedical research.

53 Here, we explored the impact of changes in data versions and analytic methods on the consistency of
54 epigenome-wide findings (**Fig 1**). We analyzed two versions of epigenetic data collected from children
55 at age 7 from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, a longitudinal
56 birth cohort near Bristol, England. We first characterized the difference between these versions with
57 respect to the distributions of DNAm at the CpG- and individual-level to illuminate the discrepancies
58 that can arise between data versions. Second, we performed two analyses to ascertain the impact of data
59 version changes at the level of CpG-associations, using classical EWAS and a more nuanced analytic
60 method called the Structured Life Course Modeling Approach (SLCMA) ⁴. We performed these
61 analyses using two different types of exposures, contrasting the results from psychosocial (childhood
62 adversity) and physical (maternal smoking during pregnancy) exposures ^{5,6}. Finally, we compared
63 results derived from SLCMA between two analytic versions, as more recent guidelines have emerged
64 on its use in big data settings ³. Overall, these analyses provide insight into the reproducibility of
65 epigenome-wide associations and highlight the features of epigenetic data that are more reproducible
66 and robust.

67

68 **MATERIALS AND METHODS**

69 **ALSPAC cohort**

70 ALSPAC is a large prospective cohort study that recruited 14,541 pregnancies in Avon, UK, with
71 expected dates of delivery between 1 April 1991 and 31 December 1992 ^{7,8}. Further details of the study
72 and available data are provided on the study website through a fully searchable data dictionary

73 (<http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/>). Please note that the study
74 website contains details of all the data that is available through a fully searchable data dictionary and
75 variable search tool (<http://www.bristol.ac.uk/alspac/researchers/our-data/>). Ethical approval for the
76 study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics
77 Committees. Consent for biological samples has been collected in accordance with the Human Tissue
78 Act (2004). Informed consent for the use of data collected via questionnaires and clinics was obtained
79 from participants following the recommendations of the ALSPAC Ethics and Law Committee at the
80 time. All data are available by request from the ALSPAC Executive Committee for researchers who
81 meet the criteria for access to confidential data (<http://www.bristol.ac.uk/alspac/researchers/access/>).

82

83 **Epigenetic data generation**

84 DNAm profiles at birth, 7, and 15 years of age are part of the Accessible Resource for Integrated
85 Epigenomic Studies (ARIES), a subsample of 1018 mother–child pairs from the ALSPAC cohort⁹. In
86 this study, we focus on the samples collected at age 7. Briefly, DNA was extracted from peripheral
87 blood samples according to established procedures. DNAm was then measured at 485,577 CpG sites
88 across the genome using the Illumina Infinium Human Methylation 450K BeadChip microarray
89 (Illumina, San Diego, CA). We received two versions of the DNAm data, which were processed using
90 different pipelines by ALSPAC, as described below.

91

92 **Epigenetic data versions**

93 In the first version, which we refer to as the *old data* (2015 version), DNAm data were processed using
94 the pipeline developed by Touleimat and Tost^{9,10}. This pipeline involved performing background
95 correction and quantile normalization using the R-package *wateRmelon*. DNAm values for all 485,577
96 CpGs were provided in the old data version.

97 In the second data version, which we refer to as the *new data* (2018 version), DNAm data were
98 processed using the pipeline developed by Min and colleagues ¹¹. In this version, background
99 correction and functional normalization of DNAm data were performed using the R-package *meffil*. In
100 addition, samples with > 10% of CpG sites with a detection p-value > 0.01 or a bead count < 3 in >
101 10% of probes were removed. As such, there were fewer CpGs available for analysis (482,855) in the
102 new data compared to the old data (**Fig 2A**). Furthermore, due to data processing and potential removal
103 of consent for some individuals, only 948 participants overlapped between both data versions (**Fig 2A**).
104 Only singleton birth participants present in both data versions were analyzed (n=946).
105 For the current analyses, we further removed cross-hybridizing probes, polymorphic probes, and probes
106 located in sex chromosomes, as well as those probes that did not overlap between both data versions.
107 These filtering steps resulted in a list of 440,257 CpGs that were present in each data version. To
108 remove possible outliers, we winsorized the beta values (i.e., values that represent % methylation) at
109 each CpG site, setting the bottom 5% and top 5% of values to the 5th and 95th quantile, respectively.

110 **Measures of childhood adversity**

111 We investigated seven types of childhood adversity assessed between birth and age 7: experiences of
112 sexual/physical abuse, caregiver physical/emotional abuse, maternal psychopathology, financial stress,
113 family instability, one-adult households, and neighborhood disadvantage. These variables were coded
114 the same way between both the old and new datasets. For a full description of these variables, please
115 refer to Dunn and colleagues (2019), which described their coding in depth ⁵.

116 **Analyses**

117 **Epigenome-wide association study (EWAS) of childhood adversity**

118 To determine how data versions can influence the results of traditional epigenome-wide methods, we
119 performed EWAS for each of the childhood adversities described above using the old and new data
120 versions. Here, we categorized children as ‘exposed’ or ‘unexposed’ to adversity on whether they

121 experienced a given adversity between ages 0 to 7. We performed these epigenome-wide associations
122 using the *limma* package in R¹². Consistent with previous work on these exposures⁵, we included the
123 following covariates to account for potential confounding: sex, race/ethnicity, maternal age at birth,
124 maternal education, birth weight, number of previous pregnancies, maternal smoking during
125 pregnancy, and cell type proportions estimated using the Houseman method¹³. We accounted for
126 multiple-testing using the Benjamini-Hochberg method and set the false discovery rate (FDR) at 5%¹⁴.

127

128 **Structured Life Course Modeling Approach (SLCMA) of childhood adversity**

129 The SLCMA is a two-stage method that compares different life course hypotheses that describe the
130 relationship between time-dependent exposures and an outcome of interest^{4,15,16}. This method
131 simultaneously compares a set of *a priori*-specified life course hypotheses encoding time-varying
132 exposure-DNA_m relationships, such as the timing of exposure (sensitive periods), or a cumulative
133 count of exposures over time (accumulation of risk). Therefore, it provides more nuanced insights
134 about exposure mechanisms beyond the traditional analyses of exposed versus unexposed individuals.
135 Importantly, the SLCMA has been applied in multiple contexts to determine whether the timing of
136 certain exposures can influence outcomes, including psychometric measures and DNA_m^{3,17}. To
137 summarize SLCMA briefly, in the first stage, variable selection (LARS-LASSO) is used to select the
138 life course hypothesis that explains the greatest proportion of outcome variation. In the second stage,
139 post-selection inference is performed to obtain point estimates, confidence intervals, and p-values for
140 the hypothesis selected from the first stage, accounting for multiple testing burden associated with
141 testing several life course hypotheses simultaneously for each locus.

142 To assess the impact of data version changes on SLCMA results, we tested the association between
143 childhood adversity and epigenetic patterns, as previously reported by Dunn and colleagues (2019), in
144 both data versions. adjusted for the same covariates as the EWAS analyses above. We tested five

145 different life course hypotheses, including three sensitive periods hypotheses encoding exposures
146 during the following three time periods: 1) very early childhood (0-2), 2) early childhood (3-5), 3)
147 middle childhood (6-7); and two additive hypotheses: 4) total number exposures across childhood
148 (accumulation), and 5) number of exposures weighted by time (recency). Post-selection inference was
149 performed using the covariance test (*covTest*) method¹⁸. We accounted for multiple-testing at the
150 epigenome-level using the Benjamini-Hochberg method and set the FDR at 5%¹⁴.

151

152 **Analytic version updates of the SLCMA of childhood adversity**

153 To determine how updates to analytic versions influence the SLCMA results, we compared the results
154 from the new data using the analysis described above, which we refer to as the *standard analysis*, to the
155 latest recommendations for the SLCMA as described by Zhu and colleagues (2020), which we refer to
156 as the *updated analysis*. This approach differed in three major ways. First, post-selection inference was
157 performed using the selective inference method, which reduces p-value inflation compared to the
158 covariance test in high dimensional analyses^{3,19}. Second, we adjusted for covariates using the Frisch-
159 Waugh-Lovell (FWL) theorem (partitioned regression)²⁰. This method has been used in penalized
160 regression analyses and can improve the statistical power to detect differences between groups^{3,21}.
161 Third, we updated the covariates to reflect best practices in the ALSPAC cohort, swapping parental
162 occupation-based social class for maternal education. Maternal education is not only a better predictor
163 of health and DNA methylation patterns, but also has better availability and comparability in other birth
164 cohorts, allowing for more direct comparisons and integration into future meta-analyses^{22,23}.

165

166 **Sensitivity analyses of prenatal exposure to maternal smoking.**

167 Given that the associations between smoking and DNA methylation are some of the best replicated
168 findings in the EWAS field, we performed additional sensitivity analyses to contrast this physical
169 exposure to the psychosocial exposures described above. We assessed the impact of data versions on

170 the association between exposure to maternal smoking during pregnancy and epigenetic patterns, as
171 previously reported by Richmond and colleagues (2018). Following the same approach as the analyses
172 of childhood adversity, we performed an EWAS of prenatal exposure to maternal smoking in the old
173 and new data versions. Maternal smoking exposure was ascertained repeatedly in all three trimesters,
174 wherein smoking at any point was considered prenatal smoking exposure ⁶. For the SLCMA analysis,
175 we tested five separate life course hypotheses of prenatal smoking exposure: first trimester, second
176 trimester, third trimester, accumulation across all trimesters, and recency of exposure.

177

178 **RESULTS**

179 **Old and new versions of the ALSPAC data differed by several key descriptive features**

180 We first assessed the CpG- and individual-level differences between the ALSPAC data normalized
181 using the Tost pipeline (*old*) and the meffil pipeline (*new*). The genome-wide distribution of DNAm
182 values from the old data were generally shifted towards the center in the new data (**Fig 2B and 2C**).
183 CpG-level variability, assessed by the standard deviation of each CpG, was generally higher in the old
184 data (**Fig 2D**). In addition, we detected higher individual-level variability (across all CpGs) in the new
185 data than in the old data, which showed no individual-level variability due to the use of quantile
186 normalization (**Fig 2E**). Nevertheless, individual-level data were generally highly correlated between
187 data versions (mean $r=0.981$, $SD=0.003$), with no clear biases being detected in specific chromosomes
188 (**Fig 2F**). However, CpGs located in 3'UTRs showed slightly lower correlations between versions (**Fig**
189 **2G**). Estimated cell-type proportions showed only slight differences between data versions but were
190 mostly similar (**Fig 2H**).

191

192 **Epigenome-wide association study results differed between data versions**

193 To determine how data versions may impact the results from traditional EWAS, we analyzed the
194 association between each of the seven childhood adversity exposures and DNA methylation at age 7 in

195 both ALSPAC DNAm data versions. Overall, we found little concordance between data versions for
196 psychosocial exposures. In the old data, we identified one CpG at an FDR <0.05 for the abuse
197 exposure, but no significant associations for the other adversities. By contrast, using the new data, we
198 identified five CpGs at an FDR <0.05, but those were associated with exposure to financial stress.
199 Moreover, no significant CpGs overlapped between the old and new data versions (**Fig 3A**). Indeed,
200 beyond significance thresholds, the overlap of CpGs by p-value rank was somewhat low for most
201 adversities (10-40%) but remained higher than by random chance (**Fig 3B**).

202 However, for each set of top CpGs (ranked by p-values), those that overlapped between data versions
203 showed relatively good rank correlation, suggesting that some signal may be retained between data
204 versions (**Fig 3C**). Importantly, top CpGs also showed high concordance in the direction and
205 magnitude of differences in DNAm between exposed and unexposed groups (**Fig 3D**). As such, it
206 appeared that the differences introduced by changing data versions caused fluctuations in the results at
207 the level of p-value thresholds, but the results from the EWAS of childhood adversity were more
208 similar when considering p-value ranks, as well as the direction and magnitude of associations.

209

210 **Data versions also changed the results from the SLCMA**

211 To determine how data versions can influence more sensitive or complex methods beyond an EWAS,
212 we assessed the impact of data versions on the SLCMA results. Here, we identified 372 CpGs in the
213 old data and 664 CpGs in the new data at an FDR<0.05 across all seven adversities, with 52 CpGs
214 overlapping between data versions (**Table 1; Fig 3E; Tables S1, S2**). The most selected hypotheses for
215 significant CpGs were different between data versions (**Fig 3F**), as were the adversities with the most
216 hits (**Table 1**). The old data showed more associations with *very early childhood* and neighborhood
217 disadvantage, whereas the new data showed more associations with *early childhood* and financial
218 stress. However, significant CpGs generally had the same hypothesis selected across data versions,

219 with little changes in the CpGs significant in the analyses of both versions (**Fig 3G**). In addition, top
220 hits generally showed the same direction of change and similar magnitude between data versions (**Fig**
221 **3H**). These results highlight the brittleness of p-value thresholds, which result in few overlaps between
222 data versions, despite the general characteristics of these CpGs and their associations being similar
223 between data versions.

224

225 **Analytic versions altered the results from the SLCMA of childhood adversity**

226 Finally, we assessed the impact of updates to analytic versions on the results from SLCMA, as per the
227 recommendations of Zhu and colleagues (2020) using only the new data version. We first performed
228 the SLCMA analysis of the childhood adversities with the standard covariates and adjustment strategy
229 but using the selective inference method in the second stage, rather than the covariance test. However,
230 only one CpG was significant at an $FDR < 0.05$ in this analysis. As such, we performed a comparison
231 between the standard analytic version and the fully updated pipeline, which uses FWL correction and
232 updated covariates. We identified 48 CpGs at an $FDR < 0.05$ in this updated analysis, with 44
233 overlapping with results from the original pipeline in the new dataset (**Fig 4A; Table S3**). The majority
234 of significant CpGs in this new analysis were association with early childhood exposure to family
235 instability, a pattern that differed slightly from the standard version of the analysis in the new data
236 (**Table 1; Fig 4B**). All significant CpGs between analytic versions showed the same hypothesis
237 selected (**Fig 4C**). These results suggested that the reduction in power of the selective inference method
238 can potentially be offset by the use of the FWL theorem and that updates to covariates only cause
239 minor changes to the results. We also note that 4 CpGs overlapped between all analyses (old data with
240 standard analysis; new data with standard analysis; new data with updated analysis), representing the
241 associations that survived technical replication across both data and analytic versions (**Table S4**).

242

243 **Sensitivity analyses of prenatal smoke exposure showed similar results to psychosocial exposures**

244 To determine whether the impact of data and analytic version changes were limited to psychosocial
245 exposures, we performed secondary analyses of prenatal smoking exposure (**supplemental materials**).
246 While the EWAS of smoking showed more overlap and consistency between data versions than
247 psychosocial exposures (**Fig S1**), we again observed differences in terms overall concordance at the
248 level of p-values and magnitude of change. These results suggested that p-value thresholds remain
249 relatively arbitrary, even with “gold-standard” epigenetic associations. Our secondary analysis of
250 prenatal smoking exposure using the SLCMA also found some overlapping CpGs at an $FDR < 0.05$ and
251 major changes to selected hypotheses between data versions (**Fig S2**). These results further suggest that
252 SLCMA was more sensitive to fluctuations between data versions than EWAS, particularly during the
253 second step of the approach when significance was assessed. We also found few overlaps between the
254 standard and updated analytic versions of the SLCMA of prenatal smoking, suggesting that updates to
255 covariates may have different effects on the results from SLCMA depending on analysis-specific
256 confounding structures, since these effects were not observed with the childhood adversity analyses
257 (**Fig S2**).

258

259 **DISCUSSION**

260 A major challenge in conducting epigenetic analyses centers around the replicability of findings across
261 cohorts, particularly when standard practices are constantly evolving. In this study, we quantified these
262 differences, showing that even within the same dataset, updates to preprocessing pipelines and analytic
263 frameworks altered the DNA methylation loci that were associated with psychosocial and physical
264 exposures at standard p-value significance thresholds, while the magnitude of differences at these loci
265 tended to remain the same.

266 The major differences between the data versions arose from two main sources: 1) individuals added or
267 removed from the analyses due to preprocessing and withdrawal of consent for certain individuals, and
268 2) changes to the preprocessing pipeline for DNAm data. Although we accounted for this first factor by
269 only analyzing overlapping samples, we found broad differences in both CpG-level and individual-
270 level DNAm patterns that must therefore be caused by preprocessing differences. One particularly
271 striking difference was observed at the individual level, wherein the new dataset showed increased
272 variability across individuals due to the use of functional normalization, rather than quantile
273 normalization in the old dataset. Such normalization techniques provide a major technical and
274 conceptual difference in the preprocessing of DNAm data, as quantile normalization assumes that all
275 individual samples have identical distributions of DNAm across the genome²⁴. Bulk differences
276 between data versions were also apparent at the level of estimated cell-type proportions. Given that cell
277 types are estimated from the DNAm data, they may reflect broader differences between data versions,
278 which may, in turn, broadly influence the results of epigenetic analyses. Overall, no single facet of the
279 data fully reflected the changes between datasets, suggesting that a combination of sample differences
280 and normalization techniques likely leads to different results between versions.

281 As such, it is perhaps unsurprising that updates to data versions resulted in broad changes to the results
282 of both our EWAS and SCLMA of psychosocial exposures. Although these exposures may have
283 subtler effects on the epigenome, we found little reproducibility at the level of p-value thresholds and
284 ranking. By contrast, the magnitude of change between exposed and unexposed individuals was highly
285 reproducible across all CpGs in both types of analyses. For the SLCMA, we also found that hypothesis
286 selection was stable across data versions (i.e., the first stage of SLCMA), but p-values obtained from
287 post-selection inference were different (i.e., the second stage of SLCMA), further highlighting the
288 fragility of inference based on p-values across our analyses. Numerous recent reports have already
289 urged the scientific community to move away from p-values as a measure of significance and

290 reproducibility since p-values can be less than informative and sometimes misleading²⁵⁻²⁸. In
291 particular, the American Statistical Association recently outlined six important principles to avoid the
292 misuse of p-values in scientific analyses²⁹. They note that p-values are not a good measure of evidence
293 on their own, nor do they measure the size or importance of an effect. Our results show these
294 statements hold true in epigenome-wide analyses. Building from our findings and prior
295 recommendations, we urge researchers to supplement standard analyses (e.g., reporting of p-values)
296 with metrics that provide additional insight into the reproducibility and strength of associations, such as
297 their magnitude and direction of effect, and allow for better understanding of both mean and variance
298 differences within a sample³⁰.

299 When we updated the SLCMA analytic version, we observed a not only a loss of p-value significance
300 for several CpGs, but also several new associations. Given that we changed three main factors between
301 analytic versions, there are at least three possible causes for these observed differences. First, selective
302 inference is more stringent than the covariance test, which can produce inappropriately small p-values
303³. This initial difference resulted in a total loss of FDR-significant CpGs, without any changes to the
304 magnitude of associations, thus explaining the reduction in the number of significant CpGs. Second,
305 the application of the FWL theorem alongside selective inference resulted in more FDR-significant
306 CpGs. However, since the FWL theorem improves statistical power without influencing the effect
307 estimates of associations³, no new associations should arise from its application in the updated analytic
308 version, which would explain the overlapping FDR-significant CpGs between the standard and updated
309 analytic versions. Thus, the third difference – updates to covariates in the statistical model – is likely
310 responsible for the emergence of four new FDR-significant CpGs in the SLCMA of psychosocial
311 exposures. Although these differences were minor, they reflect the potential effect of moving towards
312 more appropriate covariates in epigenome-wide analyses, such as the use of maternal education rather
313 than occupation-based social class in the ALSPAC cohort. This result is contrasted in the secondary

314 analyses of prenatal smoking, where changes to covariates greatly influenced the results of the
315 analyses, highlighting that careful consideration of potential confounding is required for different types
316 of analyses.

317 In contrast to the analyses of psychosocial exposures, the EWAS of prenatal smoking, a physical
318 exposure, was relatively reproducible when using p-value thresholds. This finding was expected
319 considering that cigarette smoke has the most reproduced findings from epigenome-wide studies ^{31,32}.
320 However, the overall ranking and overlap of CpGs beyond FDR-significance remained relatively low
321 in the EWAS, resulting in similar levels as psychosocial exposures across the top 5,000 CpGs. These
322 results could potentially highlight the mechanisms by which such exposures become biologically
323 embedded. Whereas smoking exposure has not just well defined, but also targeted cellular processes
324 (i.e., implicated pathways that clear toxins from the organism), psychosocial exposures may have more
325 systemic influences, impacting a broader set of CpGs with smaller effects ^{33,34}. In addition, it is
326 possible that psychosocial exposures may have greater influences in central nervous system, rather
327 than peripheral tissues, resulting in more moderate signals from blood samples ³⁵. Of note, SLCMA
328 analyses of smoking were not well reproduced across data and analytic versions. Although these results
329 may be due to a variety of factors, a potential explanation is that smoking may not be a time-dependent
330 exposure. Life course modeling approaches lose power when hypotheses are highly correlated,
331 reducing their ability to make statistical inferences ¹⁶. As such, these broad differences between
332 versions may indicate that the SCLMA is not appropriate for an exposure such as prenatal smoking,
333 which may influence epigenetic patterns equally throughout development.

334

335 The inevitable fluctuations in epigenome-wide associations highlight the importance of tracking data
336 and analytic versions across epigenetic analyses to improve both the reproducibility and replicability of
337 findings. As a field, we should endeavor to use the most up-to-date data versions and analytic models

338 before performing analyses. This approach is particularly relevant for subtler exposures, such as
339 childhood adversity, where the epigenetic signal may require more nuanced methods due to limited
340 sample sizes. Our investigation has shown the benefit of comparing data and analytic versions in a
341 stepwise manner (i.e. that the observed differences in results can be explained step by step). Moving
342 beyond p-values as a single metric for significance appears to be a necessary first step towards
343 replicability, but p-values remain an important feature of biomedical research²⁸. We propose that
344 researchers consistently report the magnitude and direction of effects alongside p-values to provide
345 insight into their findings. Furthermore, as CpGs tend to be highly correlated, nuanced approaches that
346 go beyond statistical and effect size cutoffs can be used to gain broader insight into the biological
347 mechanisms influenced by a given exposure or disease. Such methods include those assessing
348 differentially methylated or co-methylated regions^{36,37}, or genome-wide effects, such as WGCNA and
349 other network analyses³⁸.

350

351 **CONCLUSIONS**

352 Changes to both data and analytic versions do impact results derived from epigenome-wide studies
353 using both traditional and more nuanced methods. As differences not only depend on the robustness of
354 associations, but also nuances and complexities of the analyses, our results highlight the challenges in
355 making direct comparisons between and within datasets, stressing the importance of transparency in
356 reporting these differences.

357

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382

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477 Table 1. Summary of analyses and significant CpGs

Analysis details	Data version changes				Analytic version changes	
	EWAS		SLCMA		SLCMA	
Analytic approach	Ordinary least squares		Covariance test		Selective inference	
Inference method	Standard ^a		Standard ^a		Standard ^a	FWL ^b
Covariate adjustment	Old	New	Old	New	New	
Data version						
Adversity hits^c						
Abuse (sexual or physical)	1	0	66	35	0	2
Financial stress	0	5	75	294	0	2
Family instability	0	0	25	225	0	43
Maternal psychopathology	0	0	31	73	0	0
Neighborhood disadvantage	0	0	129	20	0	0
One adult household	0	0	28	7	0	0
Parental cruelty	0	0	18	10	1	1

^a Covariate adjustment was performed using standard methods.

^b Frisch-Waugh-Lovell (FWL) theorem applied for covariate adjustment and socioeconomic position replaced with maternal education.

^c Number of associated CpGs at a false-discovery rate <0.05.

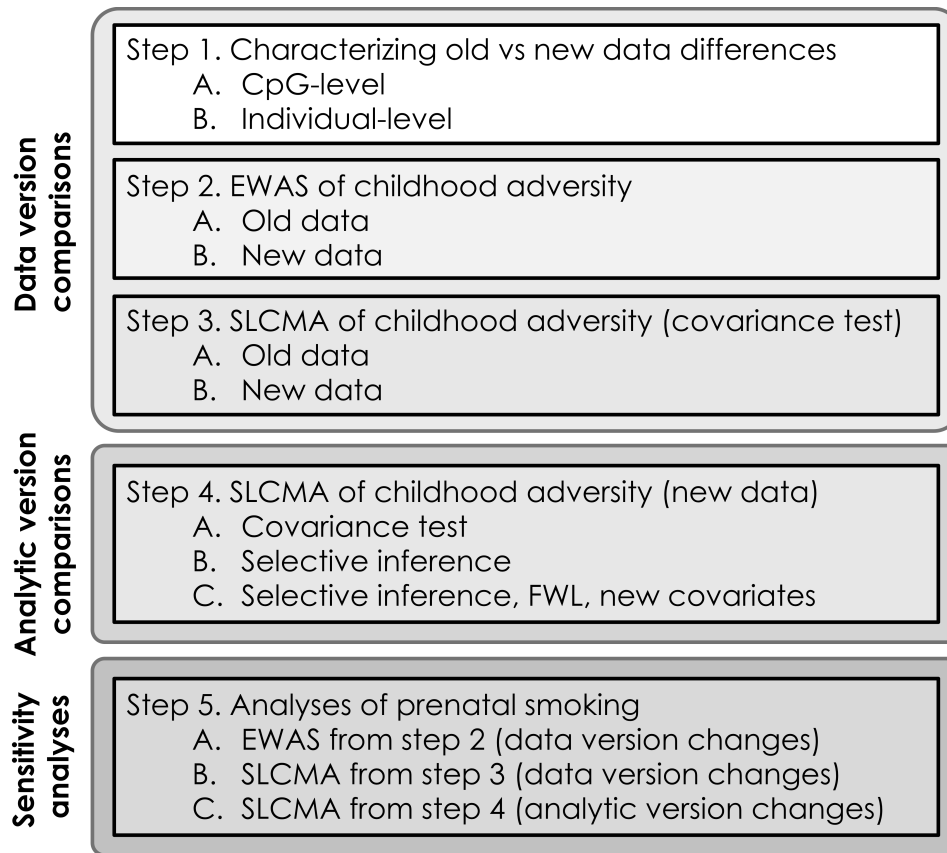


Figure 1. Overview of analyses. Steps 1-3 outline the impact of data version differences. Step 4 outlines the effect of analytic version differences. Here, childhood adversity refers to the seven different types of adversity that were assessed in these analyses. Step 5 outlines the sensitivity analyses of exposure to maternal smoking during gestation, which performed like steps 2-4. *FWL = Frisch-Waugh-Lovell theorem (covariate adjustment methods).

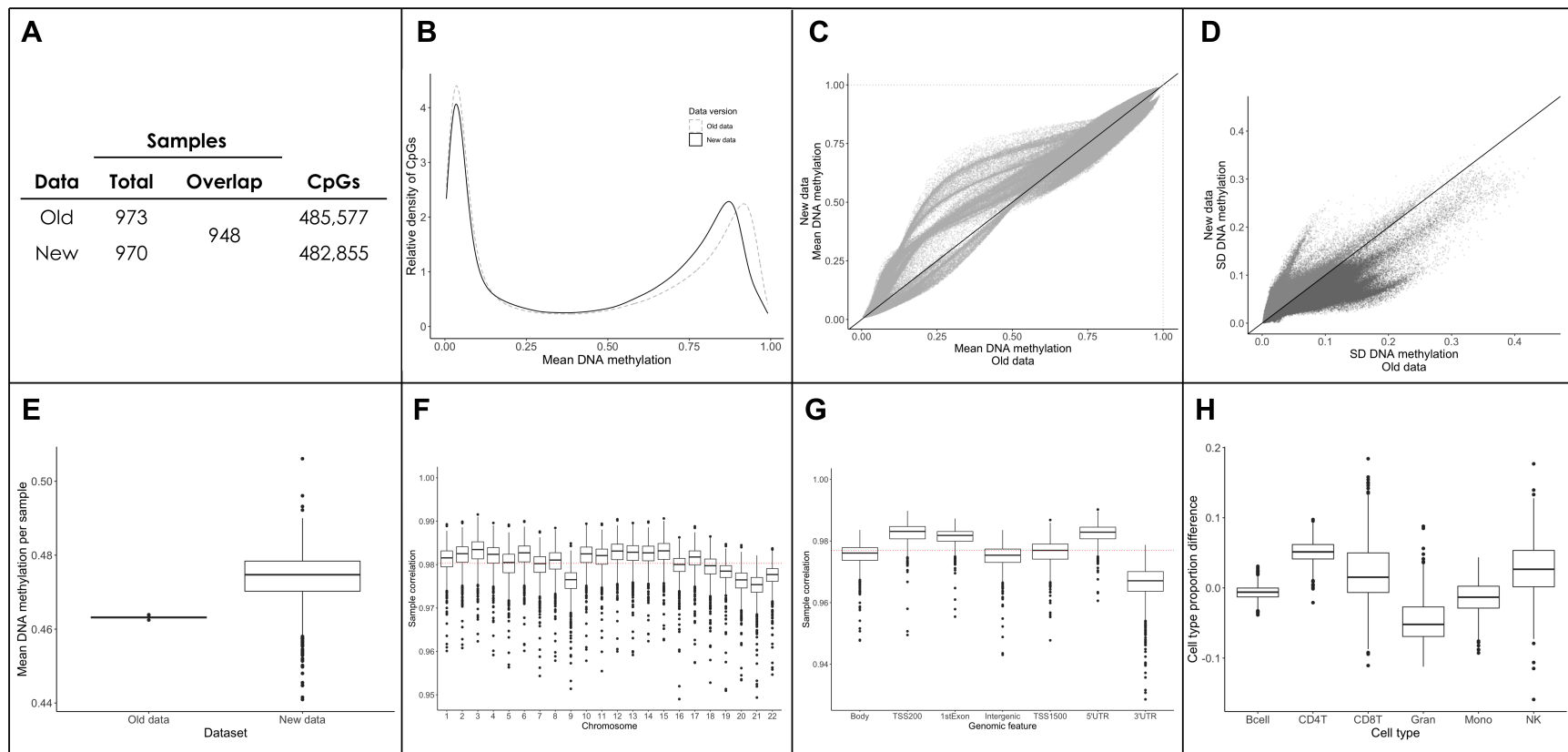


Figure 2. Differences between data versions of the ARIES cohort.

A) 948 participants overlapped between versions of the data. The new dataset had slightly less probes due to filtering procedures.

B) Both the old and the new data showed typical bimodal distributions. However, the density of genome-wide DNA methylation was shifted towards the left in the new data, suggesting that the setpoint of hypermethylated CpGs was lower in the new data.

C) Mean values for each CpG were shifted towards more middling values in the new data.

D) The standard deviation (SD) of each CpG was generally higher in the old data. 300,839 CpGs had higher variability in the old data (dark grey) and 182,016 CpGs had higher variability in the new data (light grey).

E) Individual-level mean DNA methylation (across all CpGs) varied substantially between data versions. The new data were highly variable, whereas the old data showed no variability between participants.

- F)** Individual-level DNAm data were generally highly correlated between data versions ($r=0.98$, red line), with no clear biases detected for specific chromosomes.
- G)** Individual-level DNAm from specific genomic regions were generally highly correlated between data versions ($r=0.98$, red line). However, CpGs located in 3'UTRs showed slightly lower correlations between datasets.
- H)** Estimated cell type proportions showed slight differences between the old and new datasets (differences were calculated by subtracting old data proportions from new data proportions).

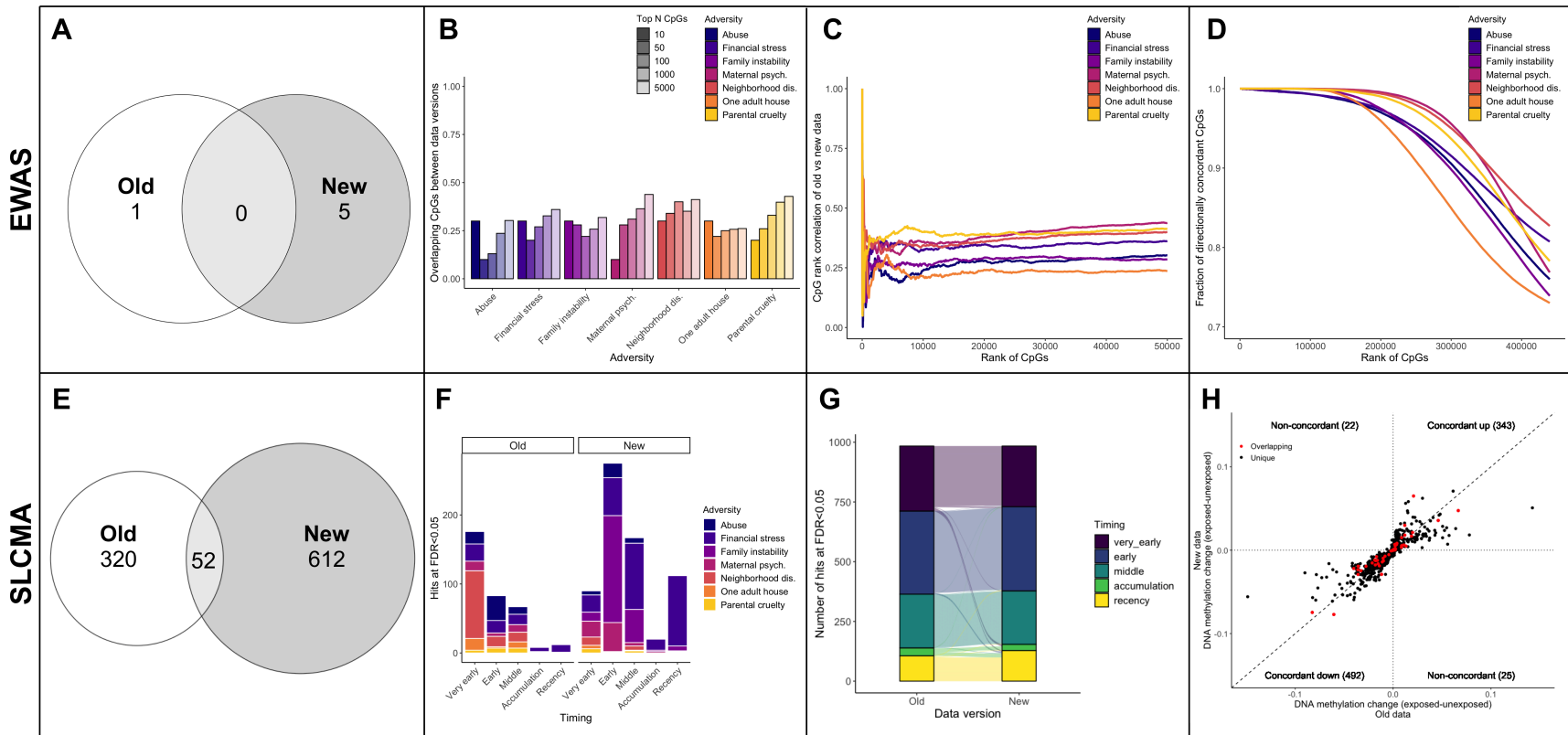


Figure 3. Updates to data versions change the results of epigenetic analyses, for both EWAS and SLCMA.

A) Overlap of the hits at $FDR < 0.05$ between the old and new data for all seven different EWAS of childhood adversity.

B) Few CpGs overlapped between the old and new data versions at different p-value rank thresholds (top 10, 50, 100, 1000, 5000, and 50000 CpGs ranked by p-value).

C) The Spearman's rank correlation between CpGs (in old versus new data) that overlapped at a given rank (i.e., top N CpGs ordered by p-value) was relatively low across both data versions.

D) The direction of DNAm differences between exposed/unexposed groups was generally consistent across overlapping CpGs at a given rank (i.e., top CpGs ranked by p-value).

E) Overlap of the hits at $FDR < 0.05$ between the old and new data for all seven different SLCMA of childhood adversity.

F) Both the hypotheses selected most frequently, and the adversities identified as having the most hits varied between data versions with the SLCMA for CpGs significant at $FDR < 0.05$.

G) The selected hypothesis from all top hits (shown in E) were generally consistent across data versions. Each line depicted corresponds to a specific CpG and shows whether its selected hypothesis differs between analyses.

H) The difference in DNAm values between exposed and unexposed participants across all top SLCMA hits from E was generally consistent between data versions, regardless of statistical significance. Only shown here are the CpGs associated with sensitive period hypotheses, as the difference between exposed and unexposed individuals is not calculated for the accumulation and recency hypotheses.

*Maternal psych = maternal psychopathology; Neighborhood dis = neighborhood disadvantage.

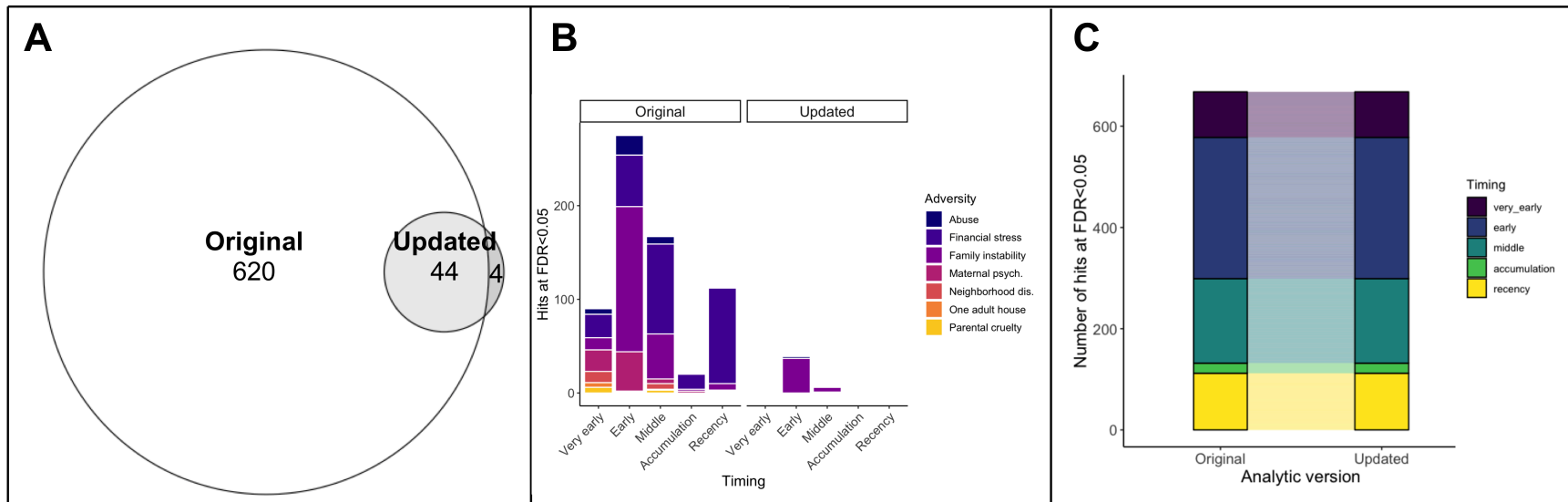


Figure 4. Updates to analytic versions change the results of SLCMA.

A) Overlap of the hits at FDR<0.05 for all seven different SLCMA of adversity between the standard and updated analytic versions (analyses performed with the new data).

B) The pattern of hypotheses selected were similar across both analytic versions, though not all adversities had statistically significant associations in the updated analytic version.

C) The hypothesis selected across all significant CpGs from A was consistent across analytic versions.

*Maternal psych = maternal psychopathology; Neighborhood dis = neighborhood disadvantage.