

***American Journal of Epidemiology* Submitted Manuscript**

Special Collection: Mental Health

Title: DNA methylation as a possible causal mechanism linking childhood adversity and health: Results from two-sample mendelian randomization study.

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Key words: Childhood adversity; Epigenetics; DNA Methylation; Mental health; Physical health; Mendelian Randomization.

Acknowledgments: This work was presented at the World Congress of Psychiatric Genetics 2023, in Montreal, October 10-14. We gratefully acknowledge our contributing studies and the participants in those studies without whom this effort would not be possible.

Funding: This work was supported by the National Institute of Mental Health of the National Institutes of Health (grant number R01MH113930 awarded to ECD). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Dr. Lussier is supported by a postdoctoral fellowship from the Canadian Institutes of Health Research (CIHR). Drs. Schuurmans was supported by a Fulbright Scholar award and by a KNAW Ter Meulen Grant from the Medical Sciences Fund of the Royal Netherlands Academy of Arts and Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The Substance Use Disorders Working Group of the Psychiatric Genomics Consortium (PGC-SUD) is supported by funds from NIDA and NIMH to MH109532. The funders took no role in the design, execution, analysis, or interpretation of the data or in the writing up of the findings.

Conflict of Interest: The authors declare no conflicts of interest.

Disclaimer: N/A

Data Availability Statement: The GWAS summary statistics used for the analysis were downloaded from publicly available sources. Summary statistics for DNAm loci were downloaded from <http://godmc.org.uk/>. Summary statistics for ADHD, anorexia nervosa, anxiety disorders, autism spectrum disorder, bipolar disorder, cannabis use disorder, obsessive-compulsive disorder, opioid exposed, post-traumatic stress disorder (PTSD), schizophrenia, and Tourette syndrome can be downloaded from <https://pgc.unc.edu/>. Summary statistics for depression can be downloaded from <https://ipsych.dk/en/research/downloads>. Summary statistics from suicide attempt can be downloaded from <https://tinyurl.com/ISGC2021>. Summary statistics for stroke, asthma, and COPD were downloaded

from <https://www.globalbiobankmeta.org/resources>. Summary statistics for chronic kidney disease can be downloaded from <http://ckdgen.imbi.uni-freiburg.de/>. Summary statistics for coronary artery disease were downloaded from <https://cvd.hugeamp.org/downloads.html#summary>. Summary statistics for obesity can be downloaded from <https://gwas.mrcieu.ac.uk/datasets/ukb-b-15541>. Summary statistics for type 2 diabetes can be downloaded from. Summary statistics for smoking initiation can be downloaded from <https://gwas.mrcieu.ac.uk/datasets/ieu-b-4877>. Summary statistics for physical inactivity were downloaded from <https://www.ebi.ac.uk/gwas/publications/36071172>. The statistical code can be downloaded from (<https://github.com/thedunnlab>).

Abstract

Childhood adversity is an important risk factor for adverse health across the life course. Epigenetic modifications, such as DNA methylation (DNAm), are one hypothesized mechanism linking adversity to disease susceptibility. Yet, few studies have determined whether adversity-related DNAm alterations are causally related to future health outcomes or if their developmental timing plays a role in these relationships. Here, we used two-sample Mendelian Randomization to obtain stronger causal inferences about the association between adversity-associated DNAm loci across development (i.e., birth; childhood; adolescence; young adulthood) and 24 mental, physical, and behavioral health outcomes. We identified particularly strong associations between adversity-associated DNAm and ADHD, depression, obsessive-compulsive disorder, suicide attempts, asthma, coronary artery disease, and chronic kidney disease. A greater number of associations were identified for birth and childhood DNAm, while adolescent and young adulthood DNAm were more closely linked to mental health. Childhood DNAm loci also showed primarily risk suppressing relationships with health outcomes, suggesting that DNAm might reflect compensatory or buffering mechanisms against childhood adversity, rather than acting solely as an indicator of disease risk. Together, our results suggest adversity-related DNAm alterations are linked to both physical and mental health outcomes, with particularly strong impacts of DNAm differences emerging earlier in development.

INTRODUCTION

Childhood adversity, such as abuse, maternal psychopathology, or poverty(1), is a significant public health concern, affecting up to two-thirds of people within the United States(2). These experiences have been linked to several negative long-term health outcomes(3, 4). For instance, people who experience four or more childhood adversities have at least two-fold higher risk for physical health problems (e.g., stroke, cardiovascular disease), five-fold higher risk for mental health problems (e.g., anxiety, depression), and six-fold higher risk for unhealthy behaviors (e.g., substance use, smoking, reduced exercise) compared to those without childhood adversities(3). While the underlying biological mechanisms linking childhood adversities to health outcomes are not yet fully understood, epigenetic modifications may be an important pathway explaining these relationships(5, 6).

Several lines of evidence suggest the association of childhood adversity with mental and physical health problems may be partially explained by epigenetic modifications, such as DNA methylation (DNAm)(7-9). DNAm is a mechanism that can tag, stabilize, or regulate genomic regions via the addition of methyl molecules to specific DNA base pairs, typically in the context of cytosine-guanine dinucleotides(10). Differences in DNAm levels may result from a complex interplay of genetic and environmental factors(11), including childhood adversity(12, 13), which may, in turn, influence downstream health outcomes. Further, recent studies using Mendelian Randomization (MR), a method that can strengthen causal inferences between exposures and outcomes by leveraging genetic variants as instrumental variables(14-16), have identified a potential causal relationship between DNAm differences and adverse health outcomes(17-21). Despite the growing evidence for a possible causal role of adversity on DNAm and subsequent health outcomes, it remains unclear whether DNAm differences *reflecting potential responses to childhood adversity* might causally influence mental and physical health outcomes. This gap limits our ability to leverage epigenetic data to predict and interpret the biological pathways underlying the wide range of health outcomes resulting from adversity.

The extent to which DNAm differences impact health outcomes may also depend on their timing, as recent evidence indicates that the relationship between childhood adversity and DNAm is not fixed but rather dynamic in nature, due to changes in the epigenome over time(22, 23). In particular, recent studies showed that epigenetic responses to childhood adversity vary across development, with different sets of loci identified in between childhood and adolescence within the same individuals(12, 13). The finding that adversity-associated DNAm differences are age-specific is suggestive of varying patterns of persistence and latency in epigenetic mechanisms, which are thought to play an important role for programming disease risk(22). However, no studies have examined the role of age-specific DNAm in the relationship between adversity, DNAm, and health outcomes. Thus, it is currently unclear whether childhood or adolescent DNAm responses to adversity are linked to similar or distinct health outcomes. If known, such insights could help determine the optimal developmental periods to leverage DNAm as a predictor for the adverse consequences of childhood adversity.

To address these gaps, we conducted a two-sample MR study of adversity-related DNAm alterations and 24 mental, physical, and behavioral health outcomes, using publicly available data from large-scale genome-wide association studies (GWAS). As we were specifically interested in age-specific DNAm profiles linked to childhood adversity, we focused on DNAm loci previously associated with childhood adversity from birth to young adulthood (age 18)(12, 13, 24, 25), rather than investigating the full epigenome. To our knowledge, this study is the first to examine the possible causal relationship between age-specific, adversity-related DNAm alterations and the mental, physical, and behavioral consequences of childhood adversity.

METHODS

Study design

In this two-sample Mendelian Randomization (MR) study, we estimated the causal relationship between DNAm and health outcomes using single nucleotide polymorphisms (SNPs) as *instrumental variables* (IV)(14-16). The underlying premise of MR is that these SNPs are related to modifiable environmental factors but are randomly distributed at the time of conception, which effectively mimics the conditions of a randomized controlled trial. The MR structure allows us to filter out the influence of unobserved confounding variables, thereby providing a less confounded estimate of the relationship between DNAm and health outcomes. **Figure S1** shows a schematic overview of the MR design.

MR analysis of DNAm-health outcome relationships is based on three key assumptions(14-16): (1) SNPs selected as IV are strongly associated with DNAm; (2) SNPs selected as IV are not associated with confounders of the association between DNAm and health outcomes; and (3) SNPs only affect health outcomes through DNAm. In other words, the instrumental variable (i.e., the SNP) is associated with the predictor variable (DNAm), but not with confounders or the outcome variable (health outcomes). These assumptions were validated as described below.

We used a *two-sample* design, using one sample to retrieve summary data for SNP-exposure associations and a second, independent, sample to retrieve summary data for the associations between SNPs and outcome. SNPs were extracted from summary statistics of publicly available, large-scale genome-wide association studies (GWAS). Ethical approval was obtained in all original studies. Reporting in this paper follows the STROBE checklist for MR studies (see **Table S1**)(26).

GWAS of DNA methylation

SNPs associated with DNAm loci were retrieved from The Genetics of DNA Methylation Consortium (GoDMC) database(27), a large-scale GWAS of DNAm data. The GoDMC database includes DNAm quantitative trait locus (mQTL) results from 32,851 European participants analyzed through SNPs associated with blood DNAm. DNAm was measured using the Infinium HumanMethylation BeadChip (HumanMethylation450 or EPIC arrays) as continuous values between 0 and 1, which represents the fraction of cells with DNAm at a given locus (e.g., percent DNAm). To adjust for confounding, each DNAm locus was corrected for age, sex, predicted cell counts, predicted smoking, genetic principal components, and potential genetic kinship. A total of 420,509 loci were available from GoDMC.

GWAS of health outcomes

We focused on health outcomes previously associated with childhood adversity(3, 4) or their psychiatric comorbidities. In total, 24 health outcomes were selected: (1) 13 mental disorders: attention-deficit hyperactivity disorder (ADHD), anorexia nervosa, anxiety disorder, autism spectrum disorder, bipolar disorder, cannabis use disorder, depression, obsessive-compulsive disorder (OCD), opioid exposed, post-traumatic stress disorder (PTSD), schizophrenia, suicide attempts, and Tourette syndrome; (2) 7 physical disorders: asthma, chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), coronary artery disease (CAD), obesity, stroke, and type 2 diabetes; and (3) 4 unhealthy behaviors: alcohol use (consumption and problems), physical inactivity (original GWAS studied physical activity, transformed here to reflect *inactivity*), and smoking initiation (ever smoked regularly yes/no). All outcomes were coded as binary variables (lifetime presence versus absence of disorder or behavior), except for alcohol use measures, which were coded continuously. Summary-level genetic data for all health outcomes were obtained from the largest publicly available GWAS (**Table 1**), all based on European samples. Sample sizes ranged from 4,503 cases and 4,173 controls for opioid dependence to 371,184 cases and 978,703 controls for depression. Health outcomes were corrected for study specific covariates (e.g., age, sex, and genetic principal components).

Primary analyses

Instrumental variable selection. We analyzed blood DNAm loci previously associated with exposure to seven types of childhood adversity in the Avon Longitudinal Study of Parents and Children (ALSPAC), including i) caregiver physical or emotional abuse; ii) sexual or physical abuse (by anyone); iii) maternal psychopathology; iv) one-adult households; v) family instability; vi) financial hardship; and vii) neighborhood disadvantage. These loci were identified from the same participants and included (1) 46 loci detected from childhood DNAm (age 7)(12) and (2) 41 different loci detected from adolescent DNAm (age 15)(13)(**Table S2**). IVs for these DNAm loci were selected in three steps. First, we identified SNPs associated with DNAm in *cis* (<1 Mb from loci; $p < 1e^{-8}$) or *trans* (>1 Mb from loci; $p < 1e^{-14}$) from GoDMC and extracted SNP-DNAm associations. Second, we extracted SNP-outcome associations from health outcome GWASs for SNPs selected in the first step. Third, we excluded SNPs with high linkage disequilibrium ($R^2 > 0.01$) or that were palindromic. Associations between IVs and DNAm are presented as standardized effect estimates (z-scores), reflecting the difference in DNAm level for each additional SNP allele. All IVs can be found **Table S3**.

Two-sample Mendelian randomization. Before conducting two-sample MR, we verified its three main assumptions(14-16). First, we analyzed mQTLs identified from a large-scale meta-analyses using stringent p-value thresholds, thereby ensuring that SNPs were strongly correlated with DNAm. Second, to limit correlations between SNPs and confounders of DNAm-health outcome relationships, we focused our analyses on individuals of European descent and adjusted each DNAm locus and health outcome for relevant covariates, including age, sex, predicted cell type proportions, smoking status, genetic principal components, and genetic kinship. Third, we investigated expression Quantitative Trait Loci (eQTLs) as a potential mechanism through which SNPs might influence health outcomes independently of mQTLs. Specifically, we employed the HELIX Web Catalogue to determine if SNPs were associated with transcriptomic or gene expression changes in blood (<https://helixomics.isglobal.org/>)(28). Finally, IVs

were cross-referenced with the GWAS catalog to identify any known associations with health outcomes (29).

We performed two-sample MR to investigate the relationships between DNAm and health outcomes, using the TwoSampleMR package(30) in R (version 4.2.2)(31). Two-sample MR analysis was run separately for each DNAm locus and health outcome. DNAm-outcome associations were estimated using the Wald method or inverse variance weighting, depending on how many SNPs were available. If no SNPs were available, the DNAm-outcome association was not analyzed (see **Table S4**). If only one SNP was available, we used Wald ratio. Wald ratio calculates the causal relationship by dividing the effect estimate of the SNP on DNAm by its effect estimate on the exposure. If multiple SNPs were available, we used inverse variance weighting (IVW). IVW analyzes the weighted average of the effect for each SNP, where the weight is the inverse of the variance of the SNP's effect estimate. The IVW method assumes all analyzed SNPs are valid IVs, and therefore provides an estimate of the overall causal effect of DNAm on the outcome. To examine the influence of age-specificity in DNAm, all analyses were presented separately for childhood DNAm loci and adolescent DNAm loci.

Given prior work showing that p-values may be unstable metrics for studying DNAm(12, 32, 33), we report associations with an uncorrected $p < 0.01$ as nominal associations. This cutoff strikes a balance between discovery and stringency, allowing for more nuanced interpretation of top findings while partially considering the number of tests conducted. To address potential issues of multiple testing, we also report p-values corrected for the number of loci tested in each health outcome using the Benjamini-Hochberg method (presented as q)(34). Associations with $q < 0.05$ were considered significant after applying the multiple test correction.

Risk increasing and suppressing role of adversity-associated DNAm. In general, adversity has a negative impact on health(3, 4) and DNAm is assumed to act on this pathway by increasing risk for

negative health outcomes (i.e., childhood adversity is linked to DNAm differences, and these DNAm differences are in turn linked to negative health outcomes)(5-9). Recent evidence, however, indicates that DNAm may also have adaptive relationships with health(35, 36), where DNAm *suppresses* the adversity-related risk for negative health outcomes, rather than solely *increasing* risk. Therefore, we assessed whether the role of adversity-related DNAm differences was to increase or suppress risk of negative health outcomes.

To investigate the role of DNAm in linking adversity to negative health, we compared the previously established associations between childhood adversity and DNAm with the associations between DNAm and individual health outcomes from this study. When adversity-DNAm and DNAm-health outcome associations were consistent (i.e., both negative or positive), the role of DNAm in linking adversity to health was *risk increasing*. By contrast, if the associations exhibited discordant directions (i.e., one positive and one negative estimate), we categorized the role of DNAm as *risk suppressing*.

Triangulation analyses

We triangulated results to investigate which findings applied across cohorts and which were specific to populations or contexts(37). By triangulating findings from different discovery sets, we could strengthen our inferences and identify more generalizable conclusions. Specifically, we investigated whether adversity-related DNAm loci identified at birth and age 18 from other studies displayed (1) comparable links with health outcomes, (2) similar age-dependent patterns, and (3) consistent roles of DNAm (i.e., risk increasing or suppressing) .

For these triangulation analyses, we utilized two different sets of DNAm loci. First, we analyzed 22 DNAm loci associated with prenatal maternal stressful event and cord blood DNAm at birth as published by a recent large-scale meta-analysis(24). Second, we investigated 39 DNAm loci previously associated with childhood victimization and blood DNAm collected in young adulthood (age 18)(25).

These triangulation sets were chosen because they were the largest available studies with comparable measures of childhood adversity to the primary studies(12, 13). The triangulation sets differed from the primary sets in three notable ways: (1) timing of DNAm measurement (birth(24) and age 18(25)); (2) tissue from which DNAm was measured (cord blood(24) and whole blood(25)); and (2) timing of childhood adversity (prenatal maternal stressors(24) and childhood sexual victimization(25)).

Sensitivity analyses

We performed 4 sets of sensitivity analyses to determine the robustness of our two-sample MR findings. For DNAm loci with two or more IVs, we report: (1) associations between DNAm and health outcomes estimated from individual SNPs using the Wald ratio; (2) directional pleiotropy calculated using MR Egger (i.e., intercept test)(38); and (3) heterogeneity test calculated using Cochran's Q-statistics(39). Finally, for DNAm loci with three or more IVs, we calculated leave-one-out estimates to identify results potentially driven by outliers.

RESULTS

Validation of MR assumptions

We observed robust associations between SNP and DNA methylation DNAm ($p < 8.2 \times 10^{-9}$), confirming the first assumption that IVs should strongly correlate with DNAm (**Table S3**). Strong effects were also indicated by magnitude of the effect estimates, which reflect the standardized difference in DNAm levels (z-score) for each additional copy of the minor SNP allele (average absolute difference=0.25, SD=0.26). In addition, no SNPs were associated with our studied health outcomes ($p < 9.0 \times 10^{-6}$; **Table S3**). Finally, DNAm was unlikely to affect health outcomes through gene expression changes, as only one mQTL (cg12023170) was also an eQTL (**Table S2**).

Associations between adversity-related DNAm loci in childhood and health outcomes

Of 46 childhood DNAm loci, 21 had associated SNPs that could be leveraged as IVs within two-sample MR. For 7 loci, we identified 2 or more SNPs (maximum 4 SNPs); for 14 loci, only 1 SNP was identified (**Table S4-S5**). Childhood DNAm loci had 16 unique associations with mental health, physical health, and unhealthy behaviors at a nominal $p < 0.01$ (**Table 2**). More specifically, we identified 6 associations between DNAm loci and *mental health outcomes* (ADHD, bipolar disorder, depression, PTSD, schizophrenia, and suicide attempts), 8 associations with *physical health outcomes* (asthma [3 loci], CAD [3 loci], CKD, and COPD), and 2 associations with *unhealthy behaviors* (alcohol consumption, problematic alcohol use). After multiple test correction, 10 associations passed the corrected $q < 0.05$ (**Figure S3**). For 75% of these associations, DNAm had a risk suppressing role (i.e., adversity-associated DNAm differences were linked to health outcomes in a way that *decreased* risk). This role was evident across mental outcomes (5/6 associations), physical outcomes (5/8 associations,) and unhealthy behaviors (2/2 associations; **Figure 1**).

Associations between adversity-related DNAm in adolescence and health outcomes

Of 41 adolescent DNAm loci, 15 had associated SNPs that could be analyzed within two-sample MR. For 6 loci, we identified 2 or more SNPs (maximum 7 SNPs) as IVs; for 9 loci, only 1 SNP was identified (**Table S4**). Overall, we identified fewer associations with adolescent DNAm loci than with childhood DNAm loci, as we identified 8 unique associations between adolescent DNAm loci and health outcomes at nominal $p < 0.01$ (**Table 3**). Relatively more associations emerged for mental than for physical health outcomes, with 5 associations to *mental health outcomes* (anorexia nervosa, bipolar disorder, OCD, Tourette syndrome, schizophrenia) and 3 to *physical health outcomes* (i.e., CKD, COPD, and stroke). Two associations met the corrected $q < 0.05$ (**Figure S2**). In contrast to childhood DNAm, only 25% of adolescent associations were risk suppressing (i.e., anorexia nervosa, CKD). For full results, see

Table S5.***Triangulation in an independent set of adversity-related loci***

We next triangulated findings using adversity-related DNAm loci identified from independent studies and datasets earlier and later in development. Because different sets of loci were identified across studies, we focused on broader patterns of replication (i.e., sets of outcomes emerging, timing of associations; risk increasing versus suppressing role of DNAm), rather than specific associations with health outcomes.

For *birth* DNAm loci related to maternal stressful events, 8 out of 22 loci could be analyzed using MR (4 loci with 2 or more SNPs associated, 4 loci had 1 SNP associated). Relative to the number of loci studied, most associations were identified for this set of DNAm loci, with 9 unique associations at nominal $p < 0.01$, including schizophrenia [2 loci], CAD, COPD, obesity, type 2 diabetes [2 loci], physical inactivity, and smoking (**Table 4**). All these associations survived multiple test correction. Similar to childhood DNAm loci, we identified more associations with physical disorders than mental outcomes. Further, 44% of birth DNAm loci had risk suppressing role.

For *young adulthood* DNAm loci related to childhood victimization, 19 of 39 loci could be analyzed using MR (11 loci had 2 or more SNPs associated, 8 loci had 1 SNP associated). Relative to the number of DNAm loci investigated, we identified the fewest associations at this developmental period. Only 11 unique associations at nominal $p < 0.01$ emerged, showing links between DNAm and anxiety disorders, bipolar disorder, OCD, PTSD, suicide attempt, asthma, CAD, CKD, COPD, alcohol consumption, and alcohol problems (**Table 4**). Five associations survived multiple test correction. Similar to our primary set of adolescent loci, more associations were detected with mental disorders and only 36% of adversity-related DNAm differences were risk suppressing. For full results, see **Figure S3** and **Table S6**.

When contrasting all four studies, we observed that early DNAm differences were more closely linked to physical disorders, while later differences showed stronger associations with mental outcomes. We also identified a greater relative number of associations between DNAm and health outcomes when DNAm was measured earlier (i.e., birth and childhood), rather than later in development. In addition, earlier DNAm differences more often had a risk suppressing role than DNAm later in life (**Figure 1**).

Sensitivity analyses

For both primary and triangulation loci, findings were comparable when results were obtained using multiple or individual IVs (**Table S7**). None of the associations showed indications of pleiotropy (MR Egger p -value<0.05) (**Table S8**). Further, no associations showed indications of heterogeneity, except for cg14855874 (adolescent locus) and schizophrenia ($p=0.022$; **Table S9**), suggesting the instruments used to test this relationship were incompatible and may be a spurious result. Finally, leave-one-out analyses showed that some associations were potentially driven by one SNP, including cg12023170 (childhood; potentially confounded by an eQTL) and CKD, cg11811897 (adolescent) and COPD, and cg25745600 (birth) on COPD and schizophrenia (**Figure S4**).

DISCUSSION

The overarching goal of this study was to obtain a stronger causal association between age-specific DNAm linked to childhood adversity and health outcomes. We highlight three key findings. First, we identified a potentially causal relationship between adversity-related DNAm differences and various health outcomes. Second, associations were age-specific, where DNAm alterations that emerged early in development (i.e., birth and childhood) had more links to health outcomes than those present in adolescence. Third, to our surprise, we found that adversity-related DNAm differences may potentially suppress the negative relationship between adversity and health, rather than increasing risk of disease.

We found that adversity-related DNAm differences were linked to various health outcomes, encompassing mental health, physical health, and unhealthy behaviors; with particularly strong findings for ADHD, depression, OCD, suicide attempts, asthma, CAD, and CKD. Interestingly, physical health outcomes were observed more often in association to birth and childhood DNAm differences, while mental health outcomes were observed more often in association to adolescent and young adulthood DNAm differences. This increased burden for mental health later in development coincides with the peak onset of many psychiatric disorder, which are centered around adolescence(40). These results suggest DNAm may play a causal role in linking *childhood adversity to future health*, which is in line with the previously hypothesized role for DNAm (7-9). While there are likely multiple other biological mechanisms at play beyond DNAm (e.g., autonomic, neuroendocrine, and immune responses(41)), our findings suggest DNAm may be one of the key players in the pathways underlying the deleterious consequences of childhood adversity.

Our study further revealed a time-sensitive role for DNAm in linking adversity to health. Specifically, DNAm differences emerging *earlier in development* were particularly important in associations to health outcomes, as we had relatively more findings for *childhood* DNAm (15 associations for 21 loci investigated) than for *adolescent* DNAm (8 associations for 15 loci). Importantly, we replicated this finding using two independent sets of DNAm loci, where we identified relatively more associations for *birth* DNAm (8 associations for 8 loci) than for *young adulthood* DNAm (9 associations for 19 loci). Previous research has already indicated that the developmental timing of DNAm differences may play a crucial role in health, particularly for *neurodevelopmental* disorders(42). For example, a prior study showed that DNAm at *birth* associates more strongly with ADHD-symptoms than DNAm measured during *childhood*(43). We extend this finding by showing that the role of DNAm continues to fade into adolescence, further pointing to early life as a *sensitive period* for DNAm in the development of adverse health problems(44). As numerous physiological systems are programmed early in life, they may be more

prone to environmental influences that shift in their developmental trajectories during this period (e.g., early programming of adiposity is linked to an increased risk of type 2 diabetes later in life(45)).

Alternatively, associations between DNAm and health outcomes may not have persisted into adolescence due to the considerable shift in DNAm patterns across development(22, 23), or become biologically embedded into alternate pathways (e.g., brain structure)(46). Nonetheless, the findings suggest childhood is a relevant and targeted developmental window for future studies investigating the role of DNAm in the manifestation of health problems across the life course.

Of particular interest, we found that adversity-associated DNAm differences often had risk-suppressing role in linking childhood adversity to health. Historically, DNAm differences have been branded as a mechanism that increases risk of adverse health outcomes (9). Yet, our findings suggest DNAm differences may reflect a mixture of mechanisms that both increase and suppress risk. DNAm alterations may reflect the balance between homeostasis and allostasis, with DNAm potentially acting as a mechanism that modulates these adaptive systems(47). A compensatory role for DNAm has been noted in recent literature. For instance, a mediation analysis showed that DNAm of the NR3C1 gene could buffer the association of maternal anxiety with children's behavioral measures, though this association was not significant after controlling for covariates(35). Evidence from a mouse model also showed DNAm alterations protected against cisplatin-induced acute kidney injury(36). Of note, in our study, the risk suppressing role of DNAm was more evident in childhood (75%) than in adolescence (25%), suggestive of a more adaptive role early in life that primes and protects an individual for their future environment. The predominantly risk suppressing role of DNAm differences in childhood may also explain the recent finding that childhood maltreatment may have a smaller association with mental problems than initially thought(48). Despite this evidence, additional research in experimental and model systems is needed to replicate these findings and determine how DNAm might promote resilience against negative health outcomes.

Top DNAm sites were implicated in pathways related to health outcomes, including the intake of low-density lipoprotein (LDL) [SORT1](49), cell-adhesion molecules and dendrite growth [SDK1](50), inflammatory responses in the brain [SBNO2](51), and adaptive immune responses [BANK1](52). Other genes were functionally related to transcriptional regulation [TCEA3, ZNF713](53, 54), cancer [FBXO43](55), cardiac myocyte hypertrophy [AKAP13](56), or functions that are not fully understood [RAB9P1]. Thus, alterations to DNAm in these genes could potentially have direct links to health. For example, cg22346081, annotated to SORT1, had a risk-suppressing association related to CAD. As SORT1 plays a key role in lipid metabolism and LDL uptake(49), DNAm differences resulting in altered SORT1 profiles could potentially serve as a protective mechanism against atherosclerosis and related cardiovascular conditions.

Our study had several strengths. First, we utilized summary statistics from publicly available GWAS with large sample sizes, allowing for greater statistical power and detection of small effect sizes. Second, our study explored multiple sets of exposures and outcomes, providing a broad picture of the relationship between adversity-associated DNAm and health outcomes across development. Third, we triangulated findings using additional discovery sets of adversity-related DNAm loci, enhancing robustness and generalizability of our findings(37). Our study also had the following limitations. First, we restricted our analyses to individuals of European descent to minimize potential bias due to confounding by genetic ancestry. Given the importance of studying the negative outcomes of adversity across broader contexts, future studies should focus on strategies or approaches to apply MR in more ancestrally diverse cohorts. Second, we had potentially overlapping samples in SNP-exposure and SNP-outcome associations for anxiety disorders, CAD, CKD, COPD, and physical inactivity (at most, 27% in SNP-exposure and 1% in SNP-outcome associations). Although these overlaps could have led to an overestimation of the observed associations(57), recent evidence suggests the actual bias resulting from overlapping samples is rather limited when sample sizes are large, as in our analyses(58). Third, as noted

in the sensitivity analyses, some estimates may represent spurious findings driven by instrument heterogeneity, single SNPs, or eQTLs, and should thus be interpreted with caution. Fourth, reverse causation is a potential concern, as we investigated early-onset disorders (i.e., autism spectrum disorder, ADHD, and asthma) that may emerge at the same time as adversity-related DNAm differences. As we could not investigate whether these health outcomes influenced adversity-related DNAm due to the unavailability of full GWAS data from GoDMC(27), we recommend researchers explore these bidirectional associations when the data become available. Finally, we tested several exposure-outcome associations, which could increase multiple test burden. However, direct p-value cutoffs may be less robust in epigenetic research(12, 32, 33), and thus we report findings using both nominal and multiple-test corrected p-values to aid in the reproducibility of our results.

Conclusions

Overall, our study provides evidence for a potential causal relationship between adversity-related DNAm differences and health outcomes. We identified more associations between health outcomes and DNAm loci emerging earlier in development, while those from adolescence and young adulthood showed fewer associations, but greater impacts on mental health outcomes. Perhaps most importantly, our findings show that, in some cases, DNAm may promote resilience to negative health outcomes, rather than increasing risk. These findings suggest that age-specific DNAm could act as a potential biomarker for the mental and physical health outcomes associated with childhood adversity.

Supplementary Information

Appendix S1

Figure S1. A schematic overview of the two-sample mendelian randomization design.

Figure S2. Effect estimates across DNAm loci and health outcomes for primary analyses.

Figure S3. Effect estimates across DNAm loci and health outcomes for triangulation analyses.

Figure S4. Leave-one-out results (beta and 95% confidence intervals) for nominal DNAm loci with >2 SNPs as instrumental variables

Appendix S2

Table S1. STROBE checklist for MR studies

Table S2. Annotated DNAm loci

Table S3. Instrumental variables

Table S4. Number of DNAm loci analyzed for each health outcome

Table S5. Associations between DNAm loci and health outcomes (full results primary analyses)

Table S6. Associations between DNAm loci and health outcomes (full results replication analyses)

Table S7. Follow-up of DNAm loci with multiple SNPs, using Wald ratio's on all single SNP

Table S8. MR Egger results to test for directional pleiotropy (intercept test) for top and nominal DNAm loci

Table S9. Cochran's Q-statistics to test for heterogeneity for top and nominal DNAm loci

References

1. Felitti VJ, Anda RF, Nordenberg D, Williamson DF, Spitz AM, Edwards VJ, Koss MP, Marks JS. Relationships of childhood abuse and household dysfunction to many of the leading causes of death in adults: The adverse childhood experiences (ACE) study. *American Journal of Preventive Medicine*. 1998;14:245-258.
2. Giano Z, Wheeler DL, Hubach RD. The frequencies and disparities of adverse childhood experiences in the US. *BMC public health*. 2020;20:1-12.
3. Nelson CA, Bhutta ZA, Harris NB, Danese A, Samara M. Adversity in childhood is linked to mental and physical health throughout life. *bmj*. 2020;371.
4. Centers for Disease Control Prevention: We can prevent childhood adversity. 2021.
5. Szyf M, Bick J. DNA methylation: a mechanism for embedding early life experiences in the genome. *Child development*. 2013;84:49-57.
6. Szyf M, McGowan P, Meaney MJ. The social environment and the epigenome. *Environmental and molecular mutagenesis*. 2008;49:46-60.
7. Smith BJ, Lussier AA, Cerutti J, Simpkin AJ, Smith ADAC, Suderman MJ, Walton E, Schaid DJ, Dunn EC. DNA methylation partially mediates the relationship between childhood adversity and depressive symptoms in adolescence. *medRxiv*. 2021.
8. Robertson KD. DNA methylation and human disease. *Nature Reviews Genetics*. 2005;6:597-610.
9. Jones MJ, Goodman SJ, Kobor MS. DNA methylation and healthy human aging. *Aging cell*. 2015;14:924-932.
10. Bird A. DNA methylation patterns and epigenetic memory. *Genes & development*. 2002;16:6-21.
11. Van Dongen J, Nivard MG, Willemsen G, Hottenga J-J, Helmer Q, Dolan CV, Ehli EA, Davies GE, Van Ijzendoorn M, Breeze CE. Genetic and environmental influences interact with age and sex in shaping the human methylome. *Nature communications*. 2016;7:11115.
12. Lussier AA, Zhu Y, Smith BJ, Simpkin AJ, Smith ADAC, Suderman MJ, Walton E, Ressler KJ, Dunn EC. Updates to data versions and analytic methods influence the reproducibility of results from epigenome-wide association studies. *Epigenetics*. 2022.
13. Lussier AA, Zhu Y, Smith BJ, Cerutti J, Simpkin AJ, Smith AD, Suderman MJ, Walton E, Relton CL, Ressler KJ. A prospective study of time-dependent childhood adversity and DNA methylation across childhood and adolescence. *MedRxiv*. 2021.
14. Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *International journal of epidemiology*. 2003;32:1-22.
15. Lawlor DA. Commentary: Two-sample Mendelian randomization: opportunities and challenges. *International journal of epidemiology*. 2016;45:908-915.
16. Relton CL, Davey Smith G. Two-step epigenetic Mendelian randomization: a strategy for establishing the causal role of epigenetic processes in pathways to disease. *International journal of epidemiology*. 2012;41:161-176.
17. Wiklund P, Karhunen V, Richmond RC, Parmar P, Rodriguez A, De Silva M, Wielscher M, Rezwani FI, Richardson TG, Veijola J. DNA methylation links prenatal smoking exposure to later life health outcomes in offspring. *Clinical epigenetics*. 2019;11:1-16.
18. Mendelson MM, Marioni RE, Joehanes R, Liu C, Hedman ÅK, Aslibekyan S, Demerath EW, Guan W, Zhi D, Yao C. Association of body mass index with DNA methylation and gene expression in blood cells and relations to cardiometabolic disease: a Mendelian randomization approach. *PLoS medicine*. 2017;14:e1002215.
19. Zhou X, Wang L, Xiao J, Sun J, Yu L, Zhang H, Meng X, Yuan S, Timofeeva M, Law PJ. Alcohol consumption, DNA methylation and colorectal cancer risk: Results from pooled cohort studies and Mendelian randomization analysis. *International Journal of Cancer*. 2022;151:83-94.

20. Jamieson E, Korologou-Linden R, Wootton RE, Guyatt AL, Battram T, Burrows K, Gaunt TR, Tobin MD, Munafò M, Smith GD. Smoking, DNA methylation, and lung function: a mendelian randomization analysis to investigate causal pathways. *The American Journal of Human Genetics*. 2020;106:315-326.
21. Jhun MA, Smith JA, Ware EB, Kardia SL, Mosley Jr TH, Turner ST, Peyser PA, Park SK. Modeling the causal role of DNA methylation in the association between cigarette smoking and inflammation in African Americans: a 2-step epigenetic Mendelian randomization study. *American journal of epidemiology*. 2017;186:1149-1158.
22. Oh ES, Petronis A. Origins of human disease: the chrono-epigenetic perspective. *Nature Reviews Genetics*. 2021;22:533-546.
23. Mulder RH, Neumann A, Cecil CA, Walton E, Houtepen LC, Simpkin AJ, Rijlaarsdam J, Heijmans BT, Gaunt TR, Felix JF. Epigenome-wide change and variation in DNA methylation in childhood: trajectories from birth to late adolescence. *Human molecular genetics*. 2021;30:119-134.
24. Kotsakis Ruehlmann A, Sammallahiti S, Cortés Hidalgo AP, Bakulski KM, Binder EB, Campbell ML, Caramaschi D, Cecil CA, Colicino E, Cruceanu C. Epigenome-wide meta-analysis of prenatal maternal stressful life events and newborn DNA methylation. *Molecular Psychiatry*. 2023:1-11.
25. Marzi SJ, Sugden K, Arseneault L, Belsky DW, Burrage J, Corcoran DL, Danese A, Fisher HL, Hannon E, Moffitt TE. Analysis of DNA methylation in young people: limited evidence for an association between victimization stress and epigenetic variation in blood. *American Journal of Psychiatry*. 2018;175:517-529.
26. Skrivankova VW, Richmond RC, Woolf BA, Yarmolinsky J, Davies NM, Swanson SA, VanderWeele TJ, Higgins JP, Timpson NJ, Dimou N. Strengthening the reporting of observational studies in epidemiology using Mendelian randomization: the STROBE-MR statement. *Jama*. 2021;326:1614-1621.
27. Min JL, Hemani G, Hannon E, Dekkers KF, Castillo-Fernandez J, Luijk R, Carnero-Montoro E, Lawson DJ, Burrows K, Suderman M. Genomic and phenotypic insights from an atlas of genetic effects on DNA methylation. *Nature genetics*. 2021;53:1311-1321.
28. Ruiz-Arenas C, Hernandez-Ferrer C, Vives-Usano M, Marí S, Quintela I, Mason D, Cadiou S, Casas M, Andrusaityte S, Gutzkow KB. Identification of autosomal cis expression quantitative trait methylation (cis eQTM) in children's blood. *Elife*. 2022;11:e65310.
29. Sollis E, Mosaku A, Abid A, Buniello A, Cerezo M, Gil L, Groza T, Güneş O, Hall P, Hayhurst J. The NHGRI-EBI GWAS Catalog: knowledgebase and deposition resource. *Nucleic acids research*. 2023;51:D977-D985.
30. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, Laurin C, Burgess S, Bowden J, Langdon R. The MR-Base platform supports systematic causal inference across the human phenome. *elife*. 2018;7:e34408.
31. R Core Team R, Team RC: R: a language and environment for statistical computing. R Foundation for Statistical Computing; 2020. 2021.
32. Amrhein V, Greenland S. Remove, rather than redefine, statistical significance. *Nature human behaviour*. 2018;2:4-4.
33. McShane BB, Gal D, Gelman A, Robert C, Tackett JL. Abandon statistical significance. *The American Statistician*. 2019;73:235-245.
34. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal statistical society: series B (Methodological)*. 1995;57:289-300.
35. Cao-Lei L, van den Heuvel MI, Huse K, Platzer M, Elgbeili G, Braeken MA, Otte RA, Witte OW, Schwab M, Van den Bergh BR. Epigenetic modifications associated with maternal anxiety during pregnancy and children's behavioral measures. *Cells*. 2021;10:2421.
36. Guo C, Pei L, Xiao X, Wei Q, Chen J-K, Ding H-F, Huang S, Fan G, Shi H, Dong Z. DNA methylation protects against cisplatin-induced kidney injury by regulating specific genes, including interferon regulatory factor 8. *Kidney international*. 2017;92:1194-1205.

37. Lawlor DA, Tilling K, Davey Smith G. Triangulation in aetiological epidemiology. *International journal of epidemiology*. 2016;45:1866-1886.
38. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. *European journal of epidemiology*. 2017;32:377-389.
39. Bowden J, Del Greco M F, Minelli C, Zhao Q, Lawlor DA, Sheehan NA, Thompson J, Davey Smith G. Improving the accuracy of two-sample summary-data Mendelian randomization: moving beyond the NOME assumption. *International journal of epidemiology*. 2019;48:728-742.
40. Solmi M, Radua J, Olivola M, Croce E, Soardo L, Salazar de Pablo G, Il Shin J, Kirkbride JB, Jones P, Kim JH. Age at onset of mental disorders worldwide: large-scale meta-analysis of 192 epidemiological studies. *Molecular psychiatry*. 2022;27:281-295.
41. Taylor SE. Mechanisms linking early life stress to adult health outcomes. *Proceedings of the National Academy of Sciences*. 2010;107:8507-8512.
42. Cecil CA, Neumann A, Walton E. Epigenetics applied to child and adolescent mental health: Progress, challenges and opportunities. *JCPP advances*. 2023;3:e12133.
43. Neumann A, Walton E, Alemany S, Cecil C, González JR, Jima DD, Lahti J, Tuominen ST, Barker ED, Binder E. Association between DNA methylation and ADHD symptoms from birth to school age: a prospective meta-analysis. *Translational psychiatry*. 2020;10:398.
44. Shonkoff JP, Boyce WT, McEwen BS. Neuroscience, molecular biology, and the childhood roots of health disparities: building a new framework for health promotion and disease prevention. *Jama*. 2009;301:2252-2259.
45. Eriksson JG. Developmental Origins of Health and Disease—from a small body size at birth to epigenetics. *Annals of medicine*. 2016;48:456-467.
46. Walton E, Baltramonaityte V, Calhoun V, Heijmans BT, Thompson PM, Cecil CA. A systematic review of neuroimaging epigenetic research: calling for an increased focus on development. *Molecular Psychiatry*. 2023;1-9.
47. McEwen BS. Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York academy of sciences*. 1998;840:33-44.
48. Baldwin JR, Wang B, Karwatowska L, Schoeler T, Tsaligopoulou A, Munafò MR, Pingault J-B. Childhood maltreatment and mental health problems: a systematic review and meta-analysis of quasi-experimental studies. *American journal of psychiatry*. 2023;appi. ajp. 20220174.
49. Kjolby M, Nielsen MS, Petersen CM. Sortilin, encoded by the cardiovascular risk gene SORT1, and its suggested functions in cardiovascular disease. *Current atherosclerosis reports*. 2015;17:1-9.
50. Rochon P-L, Theriault C, Olguin AGR, Krishnaswamy A. The cell adhesion molecule Sdk1 shapes assembly of a retinal circuit that detects localized edges. *Elife*. 2021;10:e70870.
51. Syme TE, Grill M, Hayashida E, Viengkhou B, Campbell IL, Hofer MJ. Strawberry notch homolog 2 regulates the response to interleukin-6 in the central nervous system. *Journal of Neuroinflammation*. 2022;19:126.
52. Yang J, Ren J, Yang Y, Sun J, Zhou X, Zheng S, Xuan D, Xue Y, Fan H, Zhang J. BANK1 alters B cell responses and influences the interactions between B cells and induced T regulatory cells in mice with collagen-induced arthritis. *Arthritis research & therapy*. 2018;20:1-13.
53. Labhart P, Morgan GT. Identification of novel genes encoding transcription elongation factor TFIIS (TCEA) in vertebrates: conservation of three distinct TFIIS isoforms in frog, mouse, and human. *Genomics*. 1998;52:278-288.
54. Metsu S, Rainger JK, Debacker K, Bernhard B, Rooms L, Grafodatskaya D, Weksberg R, Fombonne E, Taylor MS, Scherer SW. A CGG-Repeat Expansion Mutation in ZNF 713 Causes FRA 7 A: Association with Autistic Spectrum Disorder in Two Families. *Human mutation*. 2014;35:1295-1300.
55. Yumimoto K, Yamauchi Y, Nakayama KI. F-box proteins and cancer. *Cancers*. 2020;12:1249.

56. Appert-Collin A, Cotecchia S, Nenniger-Tosato M, Pedrazzini T, Diviani D. The A-kinase anchoring protein (AKAP)-Lbc-signaling complex mediates $\alpha 1$ adrenergic receptor-induced cardiomyocyte hypertrophy. *Proceedings of the National Academy of Sciences*. 2007;104:10140-10145.
57. Sadreev II, Elsworth BL, Mitchell RE, Paternoster L, Sanderson E, Davies NM, Millard LA, Smith GD, Haycock PC, Bowden J. Navigating sample overlap, winner's curse and weak instrument bias in Mendelian randomization studies using the UK Biobank. *MedRxiv*. 2021:2021.2006. 2028.21259622.
58. Mounier N, Kutalik Z. Bias correction for inverse variance weighting Mendelian randomization. *Genetic Epidemiology*. 2023.
59. Demontis D, Walters GB, Athanasiadis G, Walters R, Therrien K, Nielsen TT, Farajzadeh L, Voloudakis G, Bendl J, Zeng B. Genome-wide analyses of ADHD identify 27 risk loci, refine the genetic architecture and implicate several cognitive domains. *Nature genetics*. 2023;55:198-208.
60. Watson HJ, Yilmaz Z, Thornton LM, Hübel C, Coleman JR, Gaspar HA, Bryois J, Hinney A, Leppä VM, Mattheisen M. Genome-wide association study identifies eight risk loci and implicates metabo-psychiatric origins for anorexia nervosa. *Nature genetics*. 2019;51:1207-1214.
61. Otowa T, Hek K, Lee M, Byrne EM, Mirza SS, Nivard MG, Bigdeli T, Aggen SH, Adkins D, Wolen A. Meta-analysis of genome-wide association studies of anxiety disorders. *Molecular psychiatry*. 2016;21:1391-1399.
62. Grove J, Ripke S, Als TD, Mattheisen M, Walters RK, Won H, Pallesen J, Agerbo E, Andreassen OA, Anney R. Identification of common genetic risk variants for autism spectrum disorder. *Nature genetics*. 2019;51:431-444.
63. Mullins N, Forstner AJ, O'Connell KS, Coombes B, Coleman JR, Qiao Z, Als TD, Bigdeli TB, Børte S, Bryois J. Genome-wide association study of more than 40,000 bipolar disorder cases provides new insights into the underlying biology. *Nature genetics*. 2021;53:817-829.
64. Johnson EC, Demontis D, Thorgeirsson TE, Walters RK, Polimanti R, Hatoum AS, Sanchez-Roige S, Paul SE, Wendt FR, Clarke T-K. A large-scale genome-wide association study meta-analysis of cannabis use disorder. *The Lancet Psychiatry*. 2020;7:1032-1045.
65. Als TD, Kurki M, Grove J, Voloudakis G, Therrien K, Tasanko E, Nielsen TT, Naamanka J, Veerapen K, Levey D. Identification of 64 new risk loci for major depression, refinement of the genetic architecture and risk prediction of recurrence and comorbidities. *medRxiv*. 2022:2022.2008. 2024.22279149.
66. Arnold PD, Askland KD, Barlassina C, Bellodi L, Bienvenu O, Black D, Bloch M, Brentani H, Burton CL, Camarena B. Revealing the complex genetic architecture of obsessive-compulsive disorder using meta-analysis. *Molecular psychiatry*. 2018;23:1181-1181.
67. Polimanti R, Walters RK, Johnson EC, McClintick JN, Adkins AE, Adkins DE, Bacanu S-A, Bierut LJ, Bigdeli TB, Brown S. Leveraging genome-wide data to investigate differences between opioid use vs. opioid dependence in 41,176 individuals from the Psychiatric Genomics Consortium. *Molecular psychiatry*. 2020;25:1673-1687.
68. Nievergelt CM, Maihofer AX, Klengel T, Atkinson EG, Chen C-Y, Choi KW, Coleman JR, Dalvie S, Duncan LE, Gelernter J. International meta-analysis of PTSD genome-wide association studies identifies sex-and ancestry-specific genetic risk loci. *Nature communications*. 2019;10:4558.
69. Trubetskoy V, Pardiñas AF, Qi T, Panagiotaropoulou G, Awasthi S, Bigdeli TB, Bryois J, Chen C-Y, Dennison CA, Hall LS. Mapping genomic loci implicates genes and synaptic biology in schizophrenia. *Nature*. 2022;604:502-508.
70. Mullins N, Kang J, Campos AI, Coleman JR, Edwards AC, Galfalvy H, Levey DF, Lori A, Shabalin A, Starnawska A. Dissecting the shared genetic architecture of suicide attempt, psychiatric disorders, and known risk factors. *Biological psychiatry*. 2022;91:313-327.
71. Yu D, Sul JH, Tsetsos F, Nawaz MS, Huang AY, Zelaya I, Illmann C, Osiecki L, Darrow SM, Hirschtritt ME. Interrogating the genetic determinants of Tourette's syndrome and other tic disorders through genome-wide association studies. *American Journal of Psychiatry*. 2019;176:217-227.

72. Tsuo K, Zhou W, Wang Y, Kanai M, Namba S, Gupta R, Majara L, Nkambule LL, Morisaki T, Okada Y. Multi-ancestry meta-analysis of asthma identifies novel associations and highlights the value of increased power and diversity. *Cell genomics*. 2022;2.
73. Wuttke M, Köttgen A. Insights into kidney diseases from genome-wide association studies. *Nature Reviews Nephrology*. 2016;12:549-562.
74. Wain LV, Shrine N, Artigas MS, Erzurumluoglu AM, Noyvert B, Bossini-Castillo L, Obeidat Me, Henry AP, Portelli MA, Hall RJ. Genome-wide association analyses for lung function and chronic obstructive pulmonary disease identify new loci and potential druggable targets. *Nature genetics*. 2017;49:416-425.
75. Aragam KG, Jiang T, Goel A, Kanoni S, Wolford BN, Atri DS, Weeks EM, Wang M, Hindy G, Zhou W. Discovery and systematic characterization of risk variants and genes for coronary artery disease in over a million participants. *Nature Genetics*. 2022:1-13.
76. Elsworth B: Diagnoses - secondary ICD10: E66.9 Obesity, unspecified. 2018.
77. Surakka I, Wu K-H, Hornsby W, Wolford BN, Shen F, Zhou W, Huffman JE, Pandit A, Hu Y, Brumpton B. Multi-ancestry meta-analysis identifies 2 novel loci associated with ischemic stroke and reveals heterogeneity of effects between sexes and ancestries. *medRxiv*. 2022:2022.2002. 2028.22271647.
78. Xue A, Wu Y, Zhu Z, Zhang F, Kemper KE, Zheng Z, Yengo L, Lloyd-Jones LR, Sidorenko J, Wu Y. Genome-wide association analyses identify 143 risk variants and putative regulatory mechanisms for type 2 diabetes. *Nature communications*. 2018;9:2941.
79. Sanchez-Roige S, Palmer AA, Fontanillas P, Elson SL, 23andMe Research Team tSUDWGotPGC, Adams MJ, Howard DM, Edenberg HJ, Davies G, Crist RC. Genome-wide association study meta-analysis of the alcohol use disorders identification test (AUDIT) in two population-based cohorts. *American Journal of Psychiatry*. 2019;176:107-118.
80. Wang Z, Emmerich A, Pilon NJ, Moore T, Hemerich D, Cornelis MC, Mazzaferro E, Broos S, Ahluwalia TS, Bartz TM. Genome-wide association analyses of physical activity and sedentary behavior provide insights into underlying mechanisms and roles in disease prevention. *Nature genetics*. 2022;54:1332-1344.
81. Liu M, Jiang Y, Wedow R, Li Y, Brazel DM, Chen F, Datta G, Davila-Velderrain J, McGuire D, Tian C. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nature genetics*. 2019;51:237-244.

Table 1. Genome-wide association studies used to derive summary-level SNP-outcome association data for each health outcome.

Health outcomes	Sample size	Reference
<i>Mental health outcomes</i>		
ADHD	38,691 cases and 186,843 controls	Demontis, Walters (59)
Anorexia nervosa	16,992 cases and 55,525 controls	Watson, Yilmaz (60)
Anxiety disorders	18,186 cases and 17,310 controls	Otowa, Hek (61)
Autism spectrum disorder	18,382 cases and 27,969 controls	Grove, Ripke (62)
Bipolar disorder	41,917 cases and 371,549 controls	Mullins, Forstner (63)
Cannabis use disorder	14,080 cases and 369,952 controls	Johnson, Demontis (64)
Depression	371,184 cases and 978,703 controls	Als, Kurki (65)
Obsessive-compulsive disorder	2,688 cases and 7,037 controls	Arnold, Askland (66)
Opioid dependence	4,503 cases and 4,173 controls	Polimanti, Walters (67)
PTSD	23,212 cases and 151,447 controls	Nievergelt, Maihofer (68)
Schizophrenia	53,386 cases and 77,258 controls	Trubetskoy, Pardiñas (69)
Suicide attempt	26,590 cases and 492,022 controls	Mullins, Kang (70)
Tourette syndrome	4,819 cases and 9,488 controls	Yu, Sul (71)
<i>Physical health outcomes</i>		
Asthma	90,771 cases and 1,254,131 controls	Tsuo, Zhou (72)
Chronic kidney disease	41,395 cases and 439,303 controls	Wuttke and Köttgen (73)
COPD	58,559 cases and 937,358 controls	Wain, Shrine (74)
Coronary artery disease	22,233 cases and 64,762 controls	Aragam, Jiang (75)
Obesity	4,688 cases and 458,322 controls	Elsworth (76)
Stroke	34,503 cases and 1,004,879 controls	Surakka, Wu (77)
Type 2 diabetes	62,892 cases and 596,424 controls	Xue, Wu (78)
<i>Unhealthy behaviors</i>		
Alcohol use ¹	121,604 total sample (continuous) ²	Sanchez-Roige, Palmer (79)
Physical inactivity	608,595 total sample	Wang, Emmerich (80)
Smoking	311,629 cases and 321,173 controls	Liu, Jiang (81)

ADHD = attention-deficit/hyperactivity disorder; PTSD = Post-traumatic stress disorder; COPD = chronic obstructive pulmonary disease. Summary-level genetic data for these health outcomes were obtained from the largest publicly available GWAS, all of which were based on European samples

¹ Alcohol use consists of two different outcomes, 1) alcohol consumption and 2) alcohol problems.

² A continuous variable was used to determine genome wide associations.

Table 2. Associations between childhood DNAm loci and health outcomes

Health outcome	DNAm loci	Method	SNPs	Beta [95% CI]	Odds ratio [95% CI]	p	q ¹	Adversity to DNAm ²	Role DNAm ³	Sensitivity flags ⁴
<i>Mental health</i>										
ADHD	cg01023798	WR	1	0.23 [0.09, 0.36]	1.25 [1.10, 1.43]	0.001	0.015	–	Suppress risk	
Bipolar disorder	cg27639644	IVW	2	0.08 [0.02, 0.13]	1.08 [1.02, 1.14]	0.009	0.174	–	Suppress risk	
Depression	cg01023798	WR	1	0.11 [0.07, 0.16]	1.12 [1.07, 1.17]	7.6x10⁻⁷	1.5x10⁻⁵	–	Suppress risk	
PTSD	cg01654242	WR	1	-0.37 [-0.63, -0.11]	0.69 [0.53, 0.89]	0.005	0.098	–	Suppress risk	
Schizophrenia	cg01023798	WR	1	0.18 [0.06, 0.30]	1.19 [1.06, 1.35]	0.004	0.081	–	Suppress risk	
Suicide attempt	cg01023798	WR	1	0.26 [0.12, 0.39]	1.29 [1.13, 1.48]	2.7x10⁻⁴	0.005	–	Suppress risk	
<i>Physical health</i>										
Asthma	cg01023798	WR	1	-0.16 [-0.23, -0.10]	0.85 [0.79, 0.91]	1.8x10⁻⁶	3.7x10⁻⁵	–	Increase risk	
	cg01654242	WR	1	0.17 [0.08, 0.26]	1.19 [1.08, 1.30]	2.6x10⁻⁴	0.003	–	Suppress risk	
	cg13706680	WR	1	-0.14 [-0.24, -0.04]	0.87 [0.79, 0.96]	0.006	0.041	–	Increase risk	
CAD	cg10571837	WR	1	0.23 [0.10, 0.36]	1.26 [1.10, 1.43]	0.001	0.007	–	Suppress risk	
	cg20369299	WR	1	0.16 [0.05, 0.28]	1.18 [1.05, 1.32]	0.006	0.038	–	Suppress risk	
	cg22346081	WR	1	0.14 [0.08, 0.21]	1.15 [1.08, 1.23]	2.6x10⁻⁵	0.001	–	Suppress risk	
CKD	cg12023170	IVW	4	-0.04 [-0.07, -0.01]	0.96 [0.93, 0.99]	0.002	0.047	+	Suppress risk	Outlier, eQTL
COPD	cg01023798	WR	1	-0.13 [-0.23, -0.04]	0.87 [0.80, 0.96]	0.005	0.108	–	Increase risk	
<i>Unhealthy behaviors</i>										
Alcohol consumption	cg14401897	IVW	2	0.01 [0.00, 0.02]		0.005	0.095	–	Suppress risk	
Alcohol problems	cg13817046	WR	1	0.04 [0.01, 0.06]		0.007	0.134	–	Suppress risk	

Note. CI = confidence interval; IVW = inverse variance weighting; WR = Wald ratio; ADHD = attention-deficit hyperactivity disorder; CAD = coronary artery disease; CKD = chronic kidney disease; COPD = chronic obstructive pulmonary disease; PTSD = Post-traumatic stress disorder. Odds ratios are not available for alcohol consumption and problematic alcohol use, as these were continuous outcomes.

¹ Benjamini-Hochberg corrected p-value for the number of DNAm loci analyzed within the health outcome. **Boldfaced** estimates survived multiple test correction.

² Direction of the association between adversity and DNAm, which can be negative (–) or positive (+).

³ We assessed whether the role of DNAm (which possible results from adversity) was to increase or suppress adverse health outcome. Assuming that adversity always increases the odds for negative health outcomes, it would be expected that adversity-DNAm and DNAm-adverse health associations are in the same direction, which would indicate that the role

of DNAm is to increase risk. If the direction is discordant (i.e., associations in opposite directions) may indicate that DNAm suppresses the effect of adversity on adverse health outcomes.

⁴ Heterogeneity = result did not pass heterogeneity tests; outlier = result potentially driven by outlier SNP; pleiotropy = results did not pass pleiotropy tests; eQTL = DNAm site is an eQTL, meaning that IVs might influence health outcomes through changes in gene expression, rather than DNAm.

Table 3. Associations between adolescent DNAm loci and health outcomes

Health outcome	DNAm loci	Method	SNPs	Beta [95% CI]	Odds ratio [95% CI]	p	q ¹	Adversity to DNAm ²	Role DNAm ³	Sensitivity flags ⁴
Mental health										
Anorexia nervosa	cg06812747	WR	1	0.41 [0.13, 0.70]	1.51 [1.14, 2.01]	0.005	0.068	–	Suppress risk	
Bipolar disorder	cg06215562	WR	1	-0.22 [-0.37, -0.07]	0.81 [0.69, 0.94]	0.005	0.071	–	Increase risk	
OCD	cg06812747	WR	1	-0.91 [-1.51, -0.30]	0.40 [0.22, 0.74]	0.003	0.048	–	Increase risk	
Schizophrenia	cg14855874	IVW	6	0.07 [0.02, 0.12]	1.07 [1.02, 1.13]	0.010	0.136	+	Increase risk	Heterogeneity
Tourette syndrome	cg02810291	IVW	7	-0.16 [-0.28, -0.04]	0.85 [0.76, 0.96]	0.009	0.131	–	Increase risk	
Physical health										
CKD	cg19096460	WR	1	0.47 [0.23, 0.72]	1.61 [1.26, 2.05]	1.6x10⁻⁴	0.002	–	Suppress risk	
COPD	cg11811897	IVW	3	-0.11 [-0.19, -0.03]	0.89 [0.82, 0.97]	0.007	0.107	–	Increase risk	Outlier
Stroke	cg02810291	IVW	7	-0.06 [-0.10, -0.02]	0.94 [0.90, 0.98]	0.004	0.067	–	Increase risk	

Note. CI = confidence interval; IVW = inverse variance weighting; WR = Wald ratio; CKD = chronic kidney disease; COPD = chronic obstructive pulmonary disease; OCD = obsessive-compulsive disorder.

¹ Benjamini-Hochberg corrected p-value for the number of DNAm loci analyzed within the health outcome. **Boldfaced** estimates survived multiple test correction.

² Direction of the association between adversity and DNAm, which can be negative (–) or positive (+).

³ We assessed whether the role of DNAm (which possible results from adversity) was to increase or suppress adverse health outcome. Assuming that adversity always increases the odds for negative health outcomes, it would be expected that adversity-DNAm and DNAm-adverse health associations are in the same direction, which would indicate that the role of DNAm is to increase risk. If the direction is discordant (i.e., associations in opposite directions) may indicate that DNAm suppresses the effect of adversity on adverse health outcomes.

⁴ Heterogeneity = result did not pass heterogeneity tests; outlier = result potentially driven by outlier SNP; pleiotropy = results did not pass pleiotropy tests; eQTL = DNAm site is an eQTL, meaning that IVs might influence health outcomes through changes in gene expression, rather than DNAm.

Table 4. Associations between triangulation sets of DNAm loci and health outcomes

Health outcome	DNAm loci	Method	SNPs	Beta [95% CI]	Odds ratio [95% CI]	p	q ¹	Adversity to DNAm ²	Role DNAm ³	Sensitivity flags ⁴
Birth DNAm (Kotsakis Ruehlmann, Sammallahti (24))										
Mental health										
Schizophrenia	cg09088720	WR	1	-0.18 [-0.32, -0.05]	0.83 [0.73, 0.96]	0.009	0.035	–	Increase risk	
	cg25745600	IVW	3	-0.07 [-0.11, -0.02]	0.94 [0.90, 0.98]	0.003	0.026	–	Increase risk	Outlier
Physical health										
CAD	cg25745600	IVW	3	-0.04 [-0.06, -0.02]	0.96 [0.94, 0.98]	1.8x10 ⁻⁴	0.001	–	Increase risk	Outlier
COPD	cg04679114	WR	1	-0.25 [-0.41, -0.09]	0.78 [0.67, 0.91]	0.002	0.016	+	Supresss risk	
Obesity	cg04679114	WR	1	-0.01 [-0.01, -0.00]	0.99 [0.99, 1.00]	0.003	0.027	+	Supresss risk	
Type 2 diabetes	cg08292467	WR	1	0.24 [0.06, 0.41]	1.27 [1.06, 1.51]	0.009	0.036	–	Supresss risk	
	cg23131777	WR	1	-0.32 [-0.42, -0.22]	0.72 [0.65, 0.80]	1.7x10 ⁻¹⁰	1.3x10 ⁻⁹	–	Increase risk	
Unhealthy behaviors										
Physical inactivity	cg25745600	IVW	3	-0.02 [-0.04, -0.01]	0.98 [0.96, 0.99]	0.004	0.030	–	Increase risk	
Smoking	cg04679114	WR	1	-0.01 [-0.01, -0.00]	0.99 [0.99, 1.00]	0.003	0.027	+	Supresss risk	
Adolescent DNAm (Marzi, Sugden (25))										
Mental health										
Anxiety disorders	cg18673377	IVW	5	-0.19 [-0.32, -0.06]	0.83 [0.73, 0.94]	0.003	0.057	–	Increase risk	
Bipolar disorder	cg13417559	IVW	2	-0.11 [-0.19, -0.04]	0.89 [0.83, 0.96]	0.002	0.033	+	Supresss risk	
OCD	cg07415373	IVW	6	0.21 [0.08, 0.34]	1.24 [1.09, 1.41]	0.001	0.027	–	Supresss risk	
PTSD	cg03624528	IVW	2	-0.08 [-0.14, -0.03]	0.92 [0.87, 0.97]	0.005	0.083	–	Increase risk	
Suicide attempt	cg10491628	IVW	2	-0.41 [-0.57, -0.25]	0.66 [0.57, 0.78]	5.4x10 ⁻⁷	9.7x10 ⁻⁶	+	Supresss risk	
Physical health										
Asthma	cg13431226	WR	1	-0.14 [-0.24, -0.04]	0.87 [0.79, 0.96]	0.005	0.098	–	Increase risk	
CAD	cg05406868	WR	1	-0.45 [-0.55, -0.35]	0.64 [0.57, 0.70]	4.5x10 ⁻¹⁹	8.2x10 ⁻¹⁸	–	Increase risk	
CKD	cg05233289	WR	1	-0.10 [-0.17, -0.04]	0.90 [0.84, 0.96]	0.003	0.046	–	Increase risk	
Unhealthy behaviors										

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Alcohol consumption	cg06317056	WR	1	0.01 [0.00, 0.02]	0.008	0.136	+	Increase risk
Alcohol problems	cg13417559	IVW	2	-0.01 [-0.02, -0.00]	0.003	0.063	+	Supresss risk

Note. CI = confidence interval; IVW = inverse variance weighting; WR = Wald ratio; CAD = coronary artery disease; CKD = chronic kidney disease; COPD = chronic obstructive pulmonary disease; OCD = obsessive-compulsive disorder; PTSD = Post-traumatic stress disorder. Odds ratios are not available for alcohol consumption and problematic alcohol use, as these were continuous outcomes.

¹ Benjamini-Hochberg corrected p-value for the number of DNAm loci analyzed within the health outcome. **Boldfaced** estimates survived multiple test correction.

² Direction of the association between adversity and DNAm, which can be negative (–) or positive (+).

³ We assessed whether the role of DNAm (which possible results from adversity) was to increase or suppress adverse health outcome. Assuming that adversity always increases the odds for negative health outcomes, it would be expected that adversity-DNAm and DNAm-adverse health associations are in the same direction, which would indicate that the role of DNAm is to increase risk. If the direction is discordant (i.e., associations in opposite directions) may indicate that DNAm suppresses the effect of adversity on adverse health outcomes.

⁴ Heterogeneity = result did not pass heterogeneity tests; outlier = result potentially driven by outlier SNP; pleiotropy = results did not pass pleiotropy tests; eQTL = DNAm site is an eQTL, meaning that IVs might influence health outcomes through changes in gene expression, rather than DNAm.

Figure 1. Overview of risk suppressing and risk increasing associations of DNAm across health outcomes and timepoints of DNAm measurement. ADHD = attention-deficit/hyperactivity disorder; PTSD = Post-traumatic stress disorder; COPD = chronic obstructive pulmonary disease. We assessed whether the role of adversity-related DNAm differences was to increase or suppress adverse health outcome. The number in the tile reflects the number of significant loci associated with the health outcome. If no number is shown, only 1 locus was associated with the outcome. Health outcomes with no DNAm loci associated at $p < 0.01$ are shown in white.