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EMPIRICAL ARTICLE

CHILD DEVELOPMENT

DNA methylation variation after a parenting program for child conduct problems: Findings from a randomized controlled trial

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Abstract

This study investigated associations of the Incredible Years (IY) parenting program with children's DNA methylation. Participants were 289 Dutch children aged 3–9 years (75% European ancestry, 48% female) with above-average conduct problems. Saliva was collected 2.5 years after families were randomized to IY or care as usual (CAU). Using an intention-to-treat approach, confirmatory multiple-regression analyses revealed no significant differences between the IY and CAU groups in children's methylation levels at the NR3C1 and FKBP5 genes. However, exploratory epigenome-wide analyses revealed nine differentially methylated regions between groups, coinciding with *SLAMF1*, *MITF*, *FAM200B*, *PSD3*, *SNX31*, and *CELSR1*. The study provides preliminary evidence for associations of IY with children's salivary methylation levels and highlights the need for further research into biological outcomes of parenting programs.

Child conduct problems—characterized by irritability, defiance, aggression, and vindictiveness—are a core predictor of school dropout, substance abuse, criminality, and adult psychopathology (Kim-Cohen et al., 2003). One contributing factor in the development and maintenance of child conduct problems is coercive parenting, which is marked by the use of harsh discipline and a lack of reinforcement of positive child behavior (Patterson, 2016). As such, parenting programs that address coercive processes are a first-line approach for dealing with above-average conduct problems in young children (Gatti et al., 2019) and have been found effective in reducing parent-reported and observed child conduct problems (Leijten et al., 2018; Menting et al., 2013). Although there is growing evidence that the parenting environment may produce lasting biological alterations that affect child behavior, or in other words, become 'biologically embedded' (Nelles-McGee et al., 2022; Oliveira & Fearon, 2019), relatively little is known about the potential biological mechanisms and long-term biological effects of parenting programs that address child conduct problems.

One possible way by which parenting exerts long-term effects on children's behavior is through programming

Abbreviations: CAU, care as usual; DMR, differentially methylated region; DNAm, DNA methylation; ECBI, Eyberg Child Behavior Inventory; EWAS, epigenome-wide association study; FDR, false discovery rate; FU, 2.5-year follow-up assessment; GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenocortical; IY, Incredible Years; RCT, randomized controlled trial.

of children's stress responses. In particular, exposure to harsh parenting may act as chronic stressor, leading to persistent overactivation and eventual dysregulation of the child's hypothalamic–pituitary–adrenocortical (HPA) axis—one of the major stress systems (Laurent et al., 2016; McEwen & Wingfield, 2003; Smeekens et al., 2007; Wesarg et al., 2020). In turn, HPA axis dysregulation has been linked to conduct problems in children (Hartman et al., 2013; Marsman et al., 2008; Ruttle et al., 2011) and has been shown to partially mediate associations between adverse early environments and child conduct problems (Bernard et al., 2015). On the other hand, parenting characterized by sensitivity, secure attachment, and positive reinforcement may foster healthy HPA axis regulation (Albers et al., 2008; Gunnar, 2017; Shirtcliff et al., 2017) and thus may potentially decrease risk for conduct problems.

A potential mechanism by which parenting practices become biologically embedded to shape children's stress responses and subsequent behavior is through epigenetic processes. Broadly speaking, epigenetics refers to processes that lead to modifications in gene expression without changing the underlying DNA sequence (e.g., DNA methylation [DNAm], histone modifications, and noncoding RNA). DNAm is the most widely studied epigenetic modification, which typically involves the addition of a methyl molecule to a cytosine–guanine dinucleotide (CpG) and can functionally regulate gene expression (Aristizabal et al., 2019). (Wheater et al., 2022), and may mediate the link between genetic risk and subsequent behavior (Starnawska & Demontis, 2021). Moreover, patterns of DNAm in peripheral tissues (e.g., saliva) may be valuable biomarkers for health outcomes in children, including psychiatric, neurocognitive, and immune-related disorders (for a review, see Shanthikumar et al., 2020). Importantly, accumulating evidence suggests that the social environment can induce changes in DNAm (Szyf et al., 2008; Szyf & Bick, 2013).

Seminal work with rodent models demonstrated that impoverished maternal care by rat dams can induce lasting changes in offspring DNAm that lead to exaggerated HPA axis responses and more aggressive behavior (Weaver, 2007, 2009; Weaver et al., 2005). Growing evidence from associational studies suggests a similar mechanism may exist in humans. For example, human studies demonstrate associations between extremely low-quality parenting (i.e., child maltreatment) and DNAm levels at multiple CpG sites across the genome, including two key genes involved in regulation of the HPA axis (for a systematic review, see Cecil et al., 2020): the glucocorticoid receptor gene (*NR3C1*) and the FK506-binding protein 51 gene (*FKBP5*). Relatively less attention has been given to the potential positive effects of some parenting practices on child DNAm, although there is an increasing number of candidate gene and epigenome-wide studies that show associations of more positive parenting behaviors (e.g., maternal sensitivity and neonatal contact) with child DNAm (as reviewed by Provenzi et al., 2020). For example, higher maternal sensitivity in the early weeks of life has been linked to lower levels of *NR3C1* methylation both cross-sectionally and in later childhood (Conradt et al., 2016, 2019; Creasey et al., 2023).

More recently, evidence has emerged that enriching the parenting environment through intervention may influence children's DNAm levels (e.g., Braithwaite et al., 2023; Brody et al., 2016; Gardini et al., 2022; Hoye et al., 2019; O'Donnell et al., 2018). For example, one study found an association between the Parents as Teachers program—which improves parenting knowledge and skills—and salivary DNAm levels at one locus of *NR3C1* in children living in psychosocially at-risk situations (Gardini et al., 2022). In another study, individuals whose mothers participated in a nurse visitation program that reduces risk of child abuse showed epigenome-wide variation in DNAm compared to the control group when followed up 27 years later (O'Donnell et al., 2018). Together these findings suggest that improving parenting quality through intervention can have long-term and wide-spread effects on children's DNAm patterns, including at genes involved in HPA axis regulation. However, to our knowledge, no studies have investigated whether children's DNAm levels are influenced by parenting programs that are indicated for children presenting with above-average conduct problems.

To bridge this research gap, in the current study, we examined children's salivary DNAm levels following an indicated prevention randomized controlled trial (RCT) of the Incredible Years BASIC parenting program (IY)—one of the most established, evidence-based parenting programs for reducing child conduct problems (Leijten et al., 2018). In our main preregistered confirmatory analysis, we used a candidate gene approach to compare differences in DNAm levels between children whose families were randomly allocated to the IY prevention group and children who received care as usual (CAU). Considering the sample size and number of multiple comparisons, we selected two key candidate genes involved in the HPA axis—*NR3C1* and *FKBP5*. The selection was based on the theory that the early parenting environment may induce DNAm changes at these genes, providing a mechanism by which parenting exerts long-term effects on the child's stress system and subsequent behavior, such as conduct problems (Berretta et al., 2021; Tyrka et al., 2016). Given that DNAm was measured at the final follow-up assessment after randomization to one of the two conditions (IY vs. CAU) and an intention-to-treat approach was used in the analyses, we inferred that any between-group differences in child DNAm were related to the families' allocation to IY or CAU (McCoy, 2017). Specifically, we hypothesized that children randomized to the IY group would have lower

NR3C1 and higher *FKBP5* DNAm levels at the follow-up than those randomized to the CAU group—a profile associated with less harsh parenting, improved stress regulation, and reduced risk of psychopathology (for an overview, see Wadji et al., 2021; Zannas et al., 2016). This hypothesis was based on the theoretical assumption that by reducing harsh parenting and increasing positive parenting behavior, IY may induce changes in children's DNAm that foster better stress regulation leading to reductions in conduct problems. For completeness, preregistered exploratory analysis also investigated differences between the two groups across the methylome, which could provide biomarkers of wider prevention effects and regions of interest for further research. Additionally, in a preregistered follow-up analysis, we explored whether differences in child DNAm levels between groups were related to the long-term effects of IY on child conduct problems. Finally, in a supplementary exploratory analysis to improve our understanding of the possible mechanisms of IY, we tested whether changes in positive and negative parenting practices over the course of the RCT were associated with children's DNAm at the candidate genes and any differentially methylated regions (DMRs).

METHOD

In this preregistered study (https://aspredicted.org/RGD_ZFC), children aged 3–9 years and their parents were previously enrolled in a RCT of IY BASIC as an indicated prevention program (Chhangur et al., 2012; METC UMCU, protocol number 11–320/K; ERB FMG-UvA, record 2015-CDE-6392). Full details of the original study procedure and sample are provided elsewhere (Overbeek et al., 2020; Weeland et al., 2017). In brief, families were recruited to the RCT in two cohorts for logistical reasons (September–October 2012 and 2013) following a screening for child conduct problems completed by parents, with inclusion based on scores above 75th percentile on the Eyberg Child Behavior Inventory (ECBI; Eyberg & Pincus, 1999). Exclusion criteria were intellectual disability of the parent and/or child (IQ ≤ 70) and not mastering the Dutch language. We did not exclude children based on diagnosis of conduct-related or other psychological disorder. After a baseline assessment (November–January 2012 and 2013), families were randomly allocated to either the IY group or CAU group. Further assessments were carried out immediately after the intervention and at 4 months, 1.5 years, and 2.5 years. One participating parent from each family reported on their child's conduct problems and their own parenting at every assessment and took part in a parent–child observation at the first three assessments. Children and their teachers also reported on child conduct problems at FU. Upon parental consent, children also provided saliva samples at the 2.5-year follow-up (FU) for DNAm and genomic analyses.

Participants

The flow of participants through the RCT is shown in Figure S1. Of the original study sample of 386 children, 296 (77%) provided saliva samples for genomic and DNAm analysis at FU; of which data for 289 children passed quality control on the DNAm and genomic data. Characteristics of the final sample are shown in Table 1. The sample was fairly even in terms of child sex (52% boys, 48% girls) but not parent sex (92% mothers). Parents ranged in age from 27 to 49 years at baseline and 80% had completed higher vocational or university education. Based on the reported birth country of all four grandparents, 75% of children had European ancestry, while the remaining children had Asian, African, South American, or mixed ancestry. In terms of parent-reported conduct problems at baseline, 80% of children scored more than 1 SD above the Dutch norms for their age and gender (Weeland et al., 2018). Chi-squared and Mann–Whitney *U* tests revealed no characteristic differences between the current study sample and families that did not continue in the study in terms of child age and sex, child conduct problems, or parent age and sex (Table S1).

Intervention

IY BASIC (Webster-Stratton, 2014) is a collaborative, social learning-based parenting program designed to reduce child conduct problems by teaching parents' child-led play and reinforcement skills, effective limit-setting, and nonviolent discipline techniques. Parents took part in a 2-h group session each week for 14 weeks led by two group leaders, of which at least one was certified as an IY group leader. The sessions were attended by the parent who completed the study measures, although the other parent could also choose to join. Program fidelity was monitored through protocolled checklists of session elements and was rated 86% on average (for further details, see Overbeek et al., 2020). In the current study sample, 16 of the 140 participants who were randomized to the IY group did not attend any sessions and the remaining participants attended on average 11 sessions. To preserve the randomization, we used an intention-to-treat approach, meaning we included and retained all families randomized to IY in the analyses, which minimizes any risk of bias introduced by confounding variables.

Measures

Child DNAm

Saliva was collected by passive drool with the Oragene-DNA OG-600 container (DNA Genotek, Canada) according to the manufacturer's instructions. Genomic

TABLE 1 Descriptive statistics.

	Total sample		IY		CAU		
	n	*M* (SD)	*n*	*M* (SD)	*n*	*M* (SD)	Test statistic[a]
Child sex (% male)	289	52	140	56	149	48	$\chi^2 = 1.90$, $p = .168$
Child age							
Baseline	286	6.27 (1.30)	139	6.26 (1.35)	147	6.28 (1.26)	$U = 10{,}094.00$, $p = .861$
FU	289	9.99 (1.31)	140	10.01 (1.36)	149	9.97 (1.26)	$U = 10{,}248.50$, $p = .798$
Child conduct problems (ECBI)[b]							
Baseline	288	132.71 (19.05)	139	135.24 (20.42)	149	130.35 (17.44)	$U = 8878.00$, $p = .036$
FU	285	115.35 (25.18)	138	113.56 (24.83)	147	117.04 (25.47)	$U = 9270.00$, $p = .209$
Δ Conduct problems		−17.36		−21.68		−13.31	$F(1, 281) = 6.40$, $p = .012$
Positive parenting practices (PPI)[b]							
Baseline	288	4.79 (0.57)	139	4.81 (0.58)	149	4.78 (0.56)	$U = 10{,}620$, $p = .709$
FU	283	4.80 (0.59)	137	4.91 (0.57)	147	4.69 (0.59)	$U = 12{,}320$, $p = .001$
Δ Positive practices		0.01		0.10		−0.09	$F(1, 280) = 14.39$, $p = .0002$
Negative parenting practices (PPI)[b]							
Baseline	288	2.74 (0.58)	139	2.81 (0.58)	149	2.68 (0.58)	$U = 11{,}662$, $p = .064$
FU	283	2.41 (0.56)	137	2.38 (0.53)	147	2.45 (0.59)	$U = 9497.5$, $p = .408$
Δ Negative practices		−0.33		−0.43		−0.23	$F(1, 280) = 6.29$, $p = .013$
Parent sex (% female)	289	92	140	91	149	93	$\chi^2 = .139$, $p = .438$
Parent age							
Baseline	287	38.12 (4.66)	138	38.13 (4.53)	149	38.11 (4.74)	$U = 10{,}145.00$, $p = .846$
FU	289	41.84 (4.63)	140	41.87 (4.53)	149	41.81 (4.72)	$U = 10{,}325.00$, $p = .882$
Child ancestry (*n*)	288		139		149		$\chi^2 = .156$, $p = .816$
European		217		106		111	
Asian		4		2		2	
African		6		2		4	
South American		6		4		2	
Mixed		55		25		30	

Note: Ancestry was based on the parent-reported birth country of each child's paternal and maternal grandparents.

Abbreviations: CAU, care as usual; ECBI, Eyberg Child Behavior Inventory; FU, 2.5-year follow-up; IY, Incredible Years; PPI, Parenting Practices Inventory.

[a]Test of difference between the IY group and CAU group for each variable.

[b]Conduct problems and parenting practices were reported by the same parent at each assessment.

DNA was extracted from the saliva, bisulfite was converted with the Zymo EZ DNA methylation kit (Zymo Research, Irvine, CA, USA), and genome-wide DNAm measures were obtained with the Infinium EPIC array (850 k array; Illumina, San Diego CA, USA) using standard protocols. To reduce batch effects, samples were arranged on the array using a stratified randomization by child sex, cohort, and condition. We used the *meffil* package in R version 4.03 (Min et al., 2018) to perform quality control (see Supporting Information Methods for further details) and functional normalization to reduce technical variations (Fortin et al., 2014). Methylation was analyzed using M-values (i.e., the log2 ratio of the methylated vs. unmethylated probe intensities) as recommended by Du et al. (2010). For the candidate gene analyses, we computed mean DNAm levels at seven regions of the *NR3C1* promoter and four regulatory regions of *FKBP5*, which were selected based on reported links to the parenting environment or stress regulation in prior literature and described in further detail in Supporting Information Methods.

Child conduct problems

The primary measure of levels of child conduct problems was the intensity scale of the ECBI. It was selected because it is not only designed as a brief screening instrument that can accurately discriminate between children with and without a diagnosis of a conduct-related disorder (Rich & Eyberg, 2001) but is also a sensitive measure of change during the course of (preventive) intervention (Abrahamse et al., 2015, 2016; Eyberg & Robinson, 1983). Parents completed the 36-item ECBI on the frequency of the child's conduct problems (e.g., "has temper tantrums" and "acts defiant when told to do something") rated on a 7-point scale (1 = never to 7 = always) with higher scores reflecting higher levels of parent-reported conduct problems. Internal consistency was good at baseline ($\alpha = .84$) and excellent at FU ($\alpha = .92$).

For secondary analyses testing for inter-rater differences, three secondary measures of conduct problems were also used. These were child reports and teacher reports on the conduct problems scale of the Strengths and Difficulties Questionnaire measured only at the 2.5-year follow-up, and observed conduct problems (i.e., noncompliance and oppositional behavior) measured at baseline and 4 months after post-test with the Dyadic Parent–Child Interaction Coding System–Revised (Robinson & Eyberg, 1981) based on a 20-min parent–child play session, as described by Weeland et al. (2017).

Positive and negative parenting practices

Parents reported on their parenting practices via the Parenting Practices Inventory at all assessments (Webster-Stratton et al., 2001). A positive parenting practices score was computed by taking the mean average of items on the *Positive Verbal Discipline* scale (9 items, e.g., "discussing the problem with the child") and the *Praise and Incentives* scale (11 items, e.g., "giving a hug or compliment"). A negative parenting practices score was computed by taking the mean average of items on harsh and inconsistent discipline scale (15 items, e.g., "threatening but not punishing") and the physical punishment scale (6 items, e.g., "slapping or hitting when misbehavior occurs"). Higher scores reflected more positive or negative parenting practices, respectively. For the positive parenting practices scale, internal consistency was acceptable at baseline ($\alpha = .74$) and FU ($\alpha = .76$). Likewise, for the negative parenting practices scale, internal consistency was acceptable at baseline ($\alpha = .78$) and FU ($\alpha = .79$).

Covariates

Informed by prior literature, we controlled for variation in DNAm levels due to child age, child sex, cell heterogeneity, and population stratification in all analyses (Barfield et al., 2014; Jones et al., 2018; Ong et al., 2015; see Supporting Information Methods for details). We also included baseline ECBI scores as a covariate in all analyses of IY effects to account for baseline differences in parent-reported child conduct problems between groups. Child genotype for rs1360780 was included as covariate in the *FKBP5* analysis because of reported associations with *FKBP5* DNAm (Wiechmann et al., 2019). Technical variables (i.e., sentrix row and slide), cohort, parent sex, and parent age were also evaluated as potential covariates but were not retained in the analyses (see Supporting Information Methods for details).

Statistical analyses

The analyses were performed in R version 4.0.3 unless otherwise specified. An alpha level of 0.05 was used to assess statistical significance, and a false discovery rate (FDR) correction ($q = .05$) was applied to control for multiple comparisons (Benjamini & Hochberg, 1995). In terms of missing data, ECBI scores were missing for one child at baseline and four children at FU—these data were missing completely at random (Little's test: $\chi^2 = 59.40$, $p = .959$) and thus handled with list-wise deletion. Thus, the final sample was 288 children for the main analyses and 285 children for the follow-up analysis on associations of DNAm with parent-reported conduct problems.

Preliminary analyses

Differences in characteristics (i.e., child age and sex, parent age and sex, and child conduct problems) between the IY and CAU groups were tested using

Chi-squared and Mann–Whitney U tests in SPSS Version 28. Effects of IY on parent-reported child conduct problems in the current sample were assessed using an ANCOVA with condition as a predictor of ECBI scores at FU while controlling for baseline ECBI scores. The analyses were repeated using child reports, teacher reports, and observations of child conduct problems to assess whether outcomes differed based on the reporter. Associations among the study variables and sample characteristics were calculated using Pearson correlations, Spearman's rank correlations, χ^2 tests, and Kruskal–Wallis tests.

Candidate gene analysis

In the preregistered confirmatory analyses, separate multiple linear regression models were performed in R to test associations of condition (i.e., IY vs. CAU) with mean DNAm levels at each of the preselected regions of *NR3C1* and *FKBP5*. We used multiple-regression models as opposed to the preregistered ANCOVAs because the statistical assumptions for ANCOVA were not met. As per the preregistration, to check for possible moderating effects of child sex, we repeated the regression analyses including an interaction term for condition by sex. Similarly, we repeated the *FKBP5* analyses with rs1360780 genotype as a moderator given reported parenting–genotype interactions on *FKBP5* DNAm (Klengel et al., 2013).

Epigenome-wide analyses

For the preregistered exploratory analyses, a probe-level epigenome-wide association study (EWAS) and a region-level EWAS were performed in R to examine associations of genome-wide DNAm with IY. A probe-level EWAS—a series of linear regression models—was performed using the *meffil* package to test for associations of condition (dummy coded as 1 = IY and 2 = CAU) with children's DNAm levels at 409,248 individual CpG loci that showed interindividual variability in the study sample (i.e., at least 5% range in DNAm beta values between the 10th and 90th percentiles; Edgar et al., 2017). Surrogate variables obtained by independent surrogate variable analysis (Teschendorff et al., 2011) were also included in the models to adjust for technical confounding. To capture the dependencies between neighboring CpGs and increase in statistical power, we also performed a region-level EWAS to test for genomic regions in which DNAm levels were associated with condition using the *Dmrff* package (Suderman et al., 2018). *Dmrff* identifies DMRs based on test statistics from a probe-level EWAS by testing regions that span nominally significant probes with effects of the same sign and with at most 500 bp between consecutive sites.

Follow-up analyses

We conducted a series of follow-up analyses to explore significant findings on the associations of IY with DNAm from the region-level EWAS, which were not preregistered. Panther 17.0 (Mi et al., 2020) was used to classify the biological processes of the differentially methylated genes. Additionally, a probe-level gene ontology analysis was performed to identify enriched biological pathways using the *missMethyl* package in R (Phipson et al., 2016), which corrects for bias that can occur due to the differing number of probes per gene. To explore the relevance of the main results for gene expression, the Human Protein Atlas was used to establish the specificity of mRNA expression in different tissue types and brain regions for the differentially methylated genes (www.proteinatlas.org; Sjöstedt et al., 2020; Uhlén et al., 2015). Moreover, to gain insight into how far the main results might relate to child behavior and psychopathology, the web-based search tool IMAGE-CpG (www.han-lab.org/methylation/default/imageCpG; Braun et al., 2019) was used to assess DNAm saliva–brain correlations at loci associated with IY.

Additionally, in a preregistered follow-up analysis, we assessed whether between group differences in DNAm may be related to longer-term effects of IY on child conduct problems. Specifically, multiple linear regression models in R were used to explore associations between DNAm levels at IY-associated loci and parent-reported child conduct problems at FU. As a secondary analysis to check if the results were dependent on the reporter of conduct problems, we repeated the models with child-reported, teacher-reported, and observed child conduct problems as outcomes.

Supplementary analyses

Finally, given that improvement in parenting may be a mechanism of IY's effects on child conduct problems, we tested associations of change in parenting practices with DNAm at (1) the IY-associated DMR loci and (2) the candidate gene regions and loci, in supplementary analyses that were not preregistered. Specifically, we used a series of multiple-regression models with baseline positive or negative parenting practices and child DNAm at each locus/region as predictors, and positive or negative parenting practices at FU as the outcome. These analyses were exploratory in nature and not included in the preregistration.

RESULTS

Preliminary analyses

There was one baseline characteristic that differed between the IY and CAU groups, specifically children in the IY group had higher parent-reported conduct problems (Table 1), which was controlled for in subsequent analysis. In terms of significant associations among study variables, DNAm was negatively correlated with buccal cell proportions, while parent-reported child conduct problems at baseline and FU were correlated with one another and higher in boys at both time points (see Figure S2). The only difference between cohorts was in genetic PC1, however, there was no difference between cohorts in DNAm, condition, or child conduct problems at baseline and FU.

Parent-reported child conduct problems reduced in both the IY and CAU groups from baseline to FU, as would be expected given that conduct problems show a normative decrease across childhood (Gutman et al., 2019). However, in line with results reported in the original study, an ANCOVA revealed that parent-reported child conduct problems were reduced more in the IY group than the CAU group, $F(1, 281) = 6.40$, $p = .012$. Specifically, parents reported that children showed on average an 8.69% reduction in conduct problems between baseline and FU in the IY group, compared to a 5.26% reduction in the CAU group. Although parent-reported conduct problems were significantly correlated with child reports ($r_s = .34$, $p < .001$) and teacher reports ($r_s = .39$, $p < .001$), no group differences were found for change in child conduct problems at FU when measured with child reports, $F(1, 201) = 2.53$, $p = .11$, or teacher reports, $F(1, 201) = 0.47$, $p = .49$. However, we note that baseline parent-reported child conduct problems were included as a covariate in the models because measures of child- and teacher-reported conduct problems were not available at baseline. Measures of observed conduct problems significantly correlated with parent-reported conduct problems at baseline ($r_s = .13$, $p < .027$), but were not available for the FU at 2.5 years to test long-term changes. However, there was no significant difference between the IY and CAU groups regarding change in observed child conduct problems from baseline to 4 months after post-test, $F(1, 276) = 0.28$, $p = .598$.

In terms of changes in parenting practices, parents in the IY group reported a greater decrease in negative parenting than the CAU group from baseline to FU, $F(1, 201) = 6.29$, $p = .013$. Meanwhile, group differences were found for change in positive parenting, $F(1, 201) = 14.39$, $p = .0002$. Specifically, parent-reported positive parenting increased in the IY group but decreased in the CAU group from baseline to FU.

Candidate gene analyses

Contrary to our hypotheses, mean DNAm levels at the preselected regions of *NR3C1* and *FKBP5* did not significantly differ between children in the IY group and CAU group (Table 2; all unadjusted *p*-values > .141). For completeness, we also report the associations of condition with DNAm levels at the individual loci contained in the candidate regions, which were also nonsignificant (Table S2; all unadjusted *p*-values > .122). Moreover, the results remained nonsignificant when child sex was included as a moderator in the analyses, and when genotype was included as a moderator in the *FKBP5* analyses. Furthermore, there were no meaningful changes to the results when slide and sentrix row were included as covariates.

TABLE 2 Associations of mean DNA methylation at candidate gene regions with IY.

Gene	Region	*B*	SE	Upper CI	Lower CI	*p*	*M* DNAm (IY)	*M* DNAm (CAU)	Δ DNAm
NR3C1	1h	−6.59E-03	1.87E-02	0.03	−0.04	.725	0.05	0.05	−2.78E-04
	1c	2.41E-02	1.94E-02	0.06	−0.01	.215	0.03	0.03	−1.73E-04
	1f	6.53E-03	1.42E-02	0.03	−0.02	.646	0.03	0.03	−3.62E-04
	1b	−3.47E-03	2.77E-02	0.05	−0.06	.900	0.04	0.04	−1.04E-04
	1e	1.82E-02	2.23E-02	0.06	−0.03	.414	0.05	0.06	−1.67E-03
	1j	2.40E-04	2.02E-02	0.04	−0.04	.991	0.03	0.03	−3.27E-04
	1d	6.72E-03	1.74E-02	0.04	−0.03	.701	0.05	0.05	9.33E-05
FKBP5	Intron 7	5.38E-03	2.12E-02	−0.03	0.05	.800	0.94	0.93	5.09E-04
	Intron 5	−6.67E-02	4.53E-02	−0.16	0.02	.142	0.04	0.03	2.46E-03
	TSS	1.92E-03	1.75E-02	−0.03	0.03	.913	0.03	0.03	−6.61E-05
	Promoter	−1.13E-02	1.68E-02	−0.04	0.02	.501	0.04	0.04	−2.33E-04

Note: $N = 288$. Covariates: child age and sex, buccal cell proportions, genetic PC1 and PC2, and baseline child conduct problems (and rs1360780 for FKBP5 analyses only).

Abbreviations: Δ DNAm, difference in mean DNAm (IY − CAU); CAU, care as usual; CI, 95% confidence interval; DNAm, DNA methylation (beta-values); IY, Incredible Years.

Epigenome-wide association analyses

Probe-level EWAS

The probe-level EWAS identified 13,233 probes that were differentially methylated in children in the IY group compared to the CAU group at the nominal *p*-value. A QQ plot of the distribution of *p*-values is shown in Figure S3 and the results for the top 100 differentially methylated probes are shown in Table S3. No probes remained significantly associated with IY after FDR correction for multiple testings.

Region-level EWAS

A total of 16,929 regions were investigated in the region-level EWAS, of which 511 contained more than one CpG loci. Of the regions containing more than one CpG loci, we found 9 regions spanning 26 loci where DNAm levels significantly differed between children in the IY group and CAU group after FDR correction for multiple testings (Table 3; for full regression results of the separate loci, see Table S4). These DMRs ranged from two to four CpG loci and coincided with eight genes (*SLAMF1*, *MITF*, *FAM200B*, *PSD3*, *SNX31*, and *CELSR1*).

Follow-up analyses

Gene ontology and gene expression profiles

Table 4 contains a summary of the GO annotations and expression profiles of the eight genes that coincide with the regions that were differentially methylated in the IY group. A gene ontology analysis based on loci contained within the DMRs did not reveal enrichment for any pathways. In terms of expression profiles, five of the six protein-coding genes were found to be expressed in human brain tissues (normalized transcript expression values ≥1), with *PSD3*, in particular, showing enhanced brain expression (i.e., a fourfold higher mRNA level in brain tissue compared to the average level in all other tissues).

Brain–saliva DNAm comparison

Based on available data from IMAGE-CpG (Braun et al., 2019), DNAm levels in saliva and brain tissues were compared for 17 of the 26 loci contained within the DMRs (Table S5). DNAm levels were significantly and positively correlated between saliva and brain for only one locus, cg17886715, which is located at the downstream shore of a CpG island (chr1: 247267179–247267884) that overlaps with *ZN669*. Thus, there is not convincing evidence that differential salivary DNAm levels at the other

TABLE 3 Significant results from the region-level epigenome-wide association study showing differentially methylation regions based on condition (Incredible Years vs. care as usual).

DMR genomic location[a]	Annotated gene(s)	CpG island	*N* probes	*B*	SE	*p*	*q*
chr1:160616870–160617124	SLAMF1		4	−0.08	0.02	1.20E-07	.002
chr1:247269425–247269490		chr1:247267179–247267884 (south shore)	2	−0.15	0.03	2.37E-06	.013
chr3:69915123–69915232	MITF		3	0.15	0.03	2.30E-05	.036
chr4:15691786–15692119	FAM200B		3	−0.12	0.02	6.66E-07	.006
chr8:18744446–18744721	PSD3		3	0.23	0.06	5.49E-05	.048
ch8:101662496–101662628	SNX31	chr8:101661671–101662022 (island)	2	−0.14	0.03	1.94E-05	.036
ch12:128602646–128602726	LINC02368; LINC02369		3	−0.25	0.06	2.58E-05	.036
chr13:112837467–112837657			3	−0.20	0.04	5.09E-06	.017
chr22:46770603–46770870	CELSR1	chr22:46770433–46770871 (south shore)	3	−0.10	0.02	2.71E-05	.036

Note: $N = 288$. Covariates: child age and sex, buccal cell proportions, genetic PC1 and PC2, and baseline child conduct problems.
Abbreviation: DMR, differentially methylated region.
[a]Genome build GRCh37/hg19.

TABLE 4 Summary of GO and expression profiles for genes located in differentially methylated regions for condition (Incredible Years vs. care as usual).

Gene	Gene name	GO summary	Tissue specificity	Brain region specificity
SLAMF1	Signaling Lymphocytic Activation Molecule Family Member 1	Regulation of innate and adaptive immune response	Enriched: Lymphoid tissue	Not detected
FAM200B	Family With Sequence Similarity 200 Member B	Not characterized	Low specificity	Low specificity
MITF	Melanocyte Inducing Transcription Factor	Regulation of transcription by RNA polymerase II; cell differentiation and proliferation	Low specificity	Low specificity
CELSR1	Cadherin EGF LAG Seven-Pass G-Type Receptor 1	G-protein-coupled receptor signaling pathway; embryogenesis; central nervous system development; wound healing	Enhanced: Skin	Low specificity
LINC02368	Long Intergenic Nonprotein Coding RNA 2368	Nonprotein coding RNA	n/a	n/a
LINC02369	Long Intergenic Nonprotein Coding RNA 2369	Nonprotein coding RNA	n/a	n/a
SNX31	Sorting Nexin 31	Phosphatidylinositol binding; intracellular protein transport	Enhanced: Esophagus, urinary bladder	Low specificity
PSD3	Pleckstrin And Sec7 Domain Containing 3	Regulation of catalytic activity; regulation of ARF protein signal transduction	Enhanced: Brain	Low specificity

Note: The gene ontology (GO) summary is based on PANTHER GO biological process classifications; "tissue specificity" and "brain region specificity" indicate the specificity of mRNA expression for each gene in tissue types and brain regions, respectively, based on data from The Human Protein Atlas—specificity is categorized as enriched (normalized transcript expression values, nTPM, in a particular tissue/region at least four times any other tissue/region), enhanced (nTPM in 1–5 tissues/regions at least four times the mean of other tissue/regions), low specificity (nTPM ≥1 in at least one tissue/region but not elevated in any tissue/region), and not detected (nTPM <1 in all tissue/region/cell types).

16 loci would be paralleled with brain-based DNAm levels in our study sample.

Associations of DMR loci with child conduct problems

As shown in Table 5, there were no associations of parent-reported child conduct problems at FU with DNAm levels at the loci contained within the DMRs. Secondary analyses revealed that child-reported conduct problems associated with DNAm levels at three CpG loci (cg18881723, cg26646427, and cg26646427), teacher-reported conduct problems with DNAm levels at one locus (cg17886715), and observed conduct problems at one locus (cg18886071) before FDR correction for multiple testing but not after (Table S6). There were no meaningful changes to the results when slide and sentrix row were included as covariates.

Supplementary analyses

Change in parenting practices and candidate gene methylation

There were no significant associations of change in parent-reported negative parenting practices and mean DNAm levels at the candidate gene regions (Table S7). However, as shown in Table S8, changes in negative parenting associated with DNAm levels at three individual *NR3C1* loci (i.e., cg21209684 and cg14939152 in the 1f region, and cg18019515 in the 1c region) and two individual *FKBP5* loci (i.e., cg00862770 in the TSS and cg17030679 in the promoter) at the nominal *p*-value. These associations did not survive correction for multiple testings. There were no significant associations of change in parent-reported positive parenting practices with DNAm levels at the candidate gene regions (Table S7) or their individual CpG loci (Table S8). The results did not

TABLE 5 Associations of child conduct problems with DNAm levels at loci contained within DMRs for condition (Incredible Years vs. care as usual).

DMR location[a]	CpG	*B*	SE	Lower CI	Upper CI	*p*	*q*
chr1:160616870–160617124	cg18881723	4.22E-04	3.47E-04	−2.59E-04	1.10E-03	.225	.586
	cg18886071	7.30E-04	5.82E-04	−4.10E-04	1.87E-03	.211	.586
	cg01710351	−3.71E-04	4.06E-04	−1.17E-03	4.26E-04	.362	.785
	cg00149213	−1.71E-05	4.15E-04	−8.31E-04	7.97E-04	.967	.967
chr1:247269425–247269490	cg24104569	3.87E-04	6.00E-04	−7.89E-04	1.56E-03	.519	.836
	cg17886715	8.55E-04	4.97E-04	−1.19E-04	1.83E-03	.086	.544
chr3:69915123–69915232	cg12854020	−5.71E-04	4.57E-04	−1.47E-03	3.24E-04	.212	.586
	cg13151171	−4.67E-04	3.82E-04	−1.22E-03	2.83E-04	.223	.586
	cg07113570	5.95E-05	5.16E-04	−9.53E-04	1.07E-03	.908	.967
chr4:15691786–15692119	cg02353916	1.20E-03	7.63E-04	−2.92E-04	2.70E-03	.116	.544
	cg08358041	8.83E-05	8.84E-04	−1.64E-03	1.82E-03	.920	.967
	cg11462438	−4.38E-04	6.99E-04	−1.81E-03	9.31E-04	.531	.836
chr8:18744446–18744721	cg01573321	2.12E-03	1.38E-03	−5.84E-04	4.82E-03	.126	.544
	cg05121013	1.07E-03	5.86E-04	−8.21E-05	2.21E-03	.070	.544
	cg14408900	1.43E-03	7.33E-04	−7.54E-06	2.87E-03	.052	.544
ch8:101662496–101662628	cg07542096	−3.05E-05	4.83E-04	−9.78E-04	9.17E-04	.950	.967
	cg17808631	−2.76E-04	4.63E-04	−1.18E-03	6.30E-04	.551	.836
ch12:128602646–128602726	cg25603279	−7.38E-04	8.81E-04	−2.46E-03	9.89E-04	.403	.806
	cg16478734	−5.68E-04	5.97E-04	−1.74E-03	6.03E-04	.343	.785
	cg25756180	−9.21E-04	5.45E-04	−1.99E-03	1.47E-04	.092	.544
chr13:112837467–112837657	cg20920549	1.08E-04	3.75E-04	−6.27E-04	8.43E-04	.774	.958
	cg06130218	−1.82E-04	4.99E-04	−1.16E-03	7.96E-04	.716	.958
	cg05703258	−2.40E-04	4.32E-04	−1.09E-03	6.07E-04	.579	.836
chr22:46770603–46770870	cg10701080	−7.87E-05	4.05E-04	−8.72E-04	7.15E-04	.846	.967
	cg24968629	−7.33E-04	1.14E-03	−2.96E-03	1.49E-03	.519	.836
	cg26646427	−1.46E-04	4.44E-04	−1.02E-03	7.25E-04	.743	.958

Note: $N = 285$. Parent-reported child conduct problems were measured at the 2.5-year follow-up with Eyberg Child Behavior Inventory. Covariates: child age and sex, buccal cell proportions, and genetic PC1 and PC2.

Abbreviations: CI, 95% confidence interval; DMR, differentially methylated region.

[a]Genome build GRCh37/hg19.

meaningfully change when slide and sentrix row were included as covariates.

Change in parenting practices and DMR loci methylation

There were no significant associations of change in parent-reported positive or negative parenting practices with DNAm levels at the loci contained with the IY-associated DMRs (Table S9). The results did not meaningfully change when slide and sentrix row were included as covariates.

DISCUSSION

This study investigated variation in children's DNAm levels at the 2.5-year follow-up of an RCT of a parenting program indicated for children presenting with above-average parent-reported conduct problems (i.e., IY). Contrary to our hypotheses, we did not find any preliminary indications that IY influences children's salivary DNAm levels at *NR3C1* or *FKBP5*, specifically there were no differences between children in the IY group and CAU group in terms of DNAm levels at the seven first exons of *NR3C1* or at key regions of *FKBP5* (i.e., the promoter, the TSS, intron 5, and intron 7). However, in an exploratory epigenome-wide analyses, we did find evidence of wider associations of IY with children's salivary DNAm levels. Specifically, we found nine regions on the genome that were differentially methylated in children in the IY group compared to the CAU group. These regions coincided with six protein-coding genes (i.e., *SLAMF1*, *MITF*, *FAM200B*, *SNX31*, and *CELSR1*) and two noncoding RNA genes (i.e., *LINC02368* and *LINC02369*). However, DNAm levels at the DMRs did not associate with parent-reported child conduct problems, suggesting that differences in salivary DNAm levels after IY are unrelated to the sustained effects of IY on parent-reported child conduct problems. Additional analyses indicated no associations of DNAm levels with child conduct problems when the latter were instead measured by child self-report, teacher-report, and observation.

Since IY is shown to reduce harsh parenting and increase positive parenting (Leijten et al., 2018), we expected that children in the IY group would display a DNAm profile at the 2.5-year follow-up that theoretically reflects better regulation of the HPA axis than children in the CAU group, that is, lower *NR3C1* and higher *FKBP5* salivary DNAm levels (Binder, 2009; Weaver et al., 2007). A possible reason why we did not find these results is that the current study was an indicated prevention RCT where children were recruited based on above-average conduct problems rather than family adversity. While some studies indicate associations between conduct problems and higher *NR3C1* DNAm levels (Dadds et al., 2015; Gardini et al., 2022), other studies have demonstrated that children with conduct problems display lower *NR3C1* DNAm levels in peripheral tissues (Heinrich et al., 2015) as well as lower stress reactivity (Ruttle et al., 2011). This profile is opposite to that reported in children who have experienced harsh parenting (Parade et al., 2021; Wadji et al., 2021). Thus, different biological mechanisms may link parenting to child outcomes in children with above-average conduct problems and results may not generalize across populations. Furthermore, when taken together, the results of previous studies suggest that there may be a sensitive period in early infancy for the effects of parenting on DNAm levels at stress-related genes (Conradt et al., 2016, 2019; Creasey et al., 2023; Dall' Aglio et al., 2020), whereas, in the current study, children were of preschool age or older. Another possibility for the null results is that the EPIC array has sparse coverage of the CpG sites of *NR3C1* and *FKBP5* that have been linked to the parenting environment in prior research. For example, the EPIC array only covers one CpG in the widely studied intron 7 of *FKBP5* and does not include the analogous CpG in exon 1f of *NR3C1* that associates with maternal responsiveness and later aggression in rodent studies. As such, pyrosequencing of key regions of interest in these genes may be necessary to test similar hypotheses in future studies. Finally, worth considering is that the original study was not designed to test differences in DNAm levels. Effect sizes for environment and DNAm associations tend to be small and thus the sample may not have been large enough to provide adequate statistical power to detect significant effects. The relatively wide confidence intervals for the regression coefficients in the candidate gene analyses indeed suggest some uncertainty in the precision of the estimates. Future studies may benefit from pooling data from different cohorts to replicate the results in a larger sample.

The genome-wide analyses did provide some preliminary indication that IY is associated with DNAm, and thus potentially expression, of genes involved in fundamental biological processes (e.g., cell signaling, RNA transcription, and protein transport). Of particular interest for future research may be the finding of differential DNAm at *SLAMF1* given that this gene is involved in immune regulation. Notably, immune processes have been posited as a possible mechanism by which the parenting environment influences later physical and mental health, with inflammatory biomarkers in particular shown to relate to parenting behavior and psychopathology in prior research (Byrne et al., 2017; Yuan et al., 2019). On the other hand, in the follow-up analyses, we did not find enrichment for any particular biological processes when the differentially methylated genes were considered together. Moreover, although IY increased positive parenting practices and decreased

negative parenting practices based on parent reports, we found that DNAm levels at the DMR loci were not associated with changes in these measures. This would suggest that the group differences in DNAm were not driven specifically by changes in one of these parenting constructs. Instead, it could be that changes in both measured and unmeasured parenting practices have a more cumulative impact on children's DNAm or that the differences are driven by broader effects of IY, such as less stress in the family environment. Additionally, we did not find cross-sectional associations between DNAm levels at the DMR loci and children's conduct problems measured by different raters (i.e., parent, child, teacher, and experimenter), which is perhaps not surprising given the weak correlations found between salivary and brain DNAm levels at these loci. Additionally, in terms of potential biomarkers, there have been no reported significant associations between salivary DNAm levels at the DMR loci and phenotypes listed in the EWAS Atlas (Li et al., 2019), but there have been significant associations reported in studies using other tissues (Table S10). Not exhaustively, these include associations at multiple loci with adult smoking status, glucose and insulin homeostasis, and aging.

Strengths of this study include the randomized design, which prevents selection bias and minimizes confounding by extraneous variables; the recruitment of a sample with above-average conduct problems to maximize the efficacy of IY and reflect the population within which IY is typically used to reduce conduct problems; and the 2.5-year follow-up, which allowed investigation of longer-term intervention effects. Still, the results of this study should be interpreted conservatively because of some limitations. Foremost, children's DNAm levels were not measured at the start of the RCT so we were unable to compare changes in DNAm levels between the IY and CAU groups. Notably, in several prevention RCT studies group differences in post-randomization follow-up measures of DNAm have been interpreted as indicative of causal effects of the intervention on DNAm levels (e.g., Bleker et al., 2019; Braithwaite et al., 2023; Brody et al., 2016; Gardini et al., 2022). However, given participant attrition and differences in parent-reported conduct problems at baseline in the current study, we suggest our results are considered not as causal evidence, but as associations that provide a preliminary indication of possible IY effects on children's salivary DNAm that can motivate further research. Additionally, while IY has long-term effects on child behavior, at least as rated by parents, and thus likely influences neural processes, we are unable to investigate a possible role of brain-based changes in DNAm using saliva samples. As such, the study provides some potential biomarkers for wider associations of IY with children's biology but is limited in informing our knowledge about how IY changes child behavior. To bridge this gap, future research could combine neuroimaging and hormone measurements with peripheral measures of DNAm and make use of biologically informed polyepigenetic scores (e.g., Provençal et al., 2020).

Finally, the external validity of the results should be considered. On the one hand, the current study included children from both European and non-European ancestry in similar proportions to demographic estimates for the Netherlands. Moreover, the study was balanced in terms of child sex, and families were recruited from both urban and rural areas of the Netherlands. However, it was predominately mothers who chose to participate in the study, making it unclear how far fathers' parenting practices are associated with children's DNAm. Moreover, most parents had completed higher-level education, an indicator of higher socioeconomic status, thus limiting how far these results can be generalized to families that experience socioeconomic disadvantage, which has been associated with child conduct problems independently of parenting practices (Flouri et al., 2017).

CONCLUSION

This study suggests that the IY parenting intervention that reduces parent-reported child conduct problems is not associated with children's salivary DNAm at key regions of the *NR3C1* or *FKBP5* gene measured by the EPIC array. In particular, the results highlight that associations of parenting with child DNAm may differ in children with above-average conduct problems compared to those exposed to family adversity. However, the study is the first to demonstrate wider associations between the IY parenting program and salivary DNAm levels at several regions of the genome. Although the relevance of these results for phenotypic outcomes remains unclear, the study provides potential biomarkers for further investigation and highlights a need for future research to understand the biological mechanisms and effects of preventive interventions for child conduct problems.

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CONFLICT OF INTEREST STATEMENT

The authors have declared that they have no competing or potential conflicts of interest.

DATA AVAILABILITY STATEMENT

Data to reproduce the analyses are available from the first author upon reasonable request (Nicole Creasey, n.creasey@erasmusmc.nl). The analytic code necessary to reproduce the analyses presented in this paper is publicly accessible. Code is available at the following URL:

https://osf.io/86wjy/?view_only=567f94578faf40c8b0c4334c398a8f4d. The materials necessary to attempt to replicate the findings presented here are not publicly accessible. The analyses presented here were preregistered. The preregistration is available at the following URL: https://aspredicted.org/z89ey.pdf.

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REFERENCES

Abrahamse, M. E., Junger, M., Leijten, P. H. O., Lindeboom, R., Boer, F., & Lindauer, R. J. L. (2015). Psychometric properties of the Dutch Eyberg Child Behavior Inventory (ECBI) in a community sample and a multi-ethnic clinical sample. *Journal of Psychopathology and Behavioral Assessment*, *37*(4), 679–691. https://doi.org/10.1007/s10862-015-9482-1

Abrahamse, M. E., Junger, M., Van Wouwe, M. A. M. M., Boer, F., & Lindauer, R. J. L. (2016). Treating child disruptive behavior in high-risk families: A comparative effectiveness trial from a community-based implementation. *Journal of Child and Family Studies*, *25*(5), 1605–1622. https://doi.org/10.1007/s10826-015-0322-4

Albers, E. M., Marianne Riksen-Walraven, J., Sweep, F. C. G. J., & Weerth, C. D. (2008). Maternal behavior predicts infant cortisol recovery from a mild everyday stressor. *Journal of Child Psychology and Psychiatry*, *49*(1), 97–103. https://doi.org/10.1111/j.1469-7610.2007.01818.x

Aristizabal, M. J., Anreiter, I., Halldorsdottir, T., Odgers, C. L., McDade, T. W., Goldenberg, A., Mostafavi, S., Kobor, M. S., Binder, E. B., Sokolowski, M. B., & O'Donnell, K. J. (2019). Biological embedding of experience: A primer on epigenetics. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(38), 23261–23269. https://doi.org/10.1073/pnas.1820838116

Barfield, R. T., Almli, L. M., Kilaru, V., Smith, A. K., Mercer, K. B., Duncan, R., Klengel, T., Mehta, D., Binder, E. B., Epstein, M. P., Ressler, K. J., & Conneely, K. N. (2014). Accounting for population stratification in DNA methylation studies. *Genetic Epidemiology*, *38*(3), 231–241. https://doi.org/10.1002/gepi.21789

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B: Methodological*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bernard, K., Zwerling, J., & Dozier, M. (2015). Effects of early adversity on young children's diurnal cortisol rhythms and externalizing behavior: Cortisol and externalizing behavior. *Developmental Psychobiology*, *57*(8), 935–947. https://doi.org/10.1002/dev.21324

Berretta, E., Guida, E., Forni, D., & Provenzi, L. (2021). Glucocorticoid receptor gene (*NR3C1*) methylation during the first thousand days: Environmental exposures and developmental outcomes. *Neuroscience and Biobehavioral Reviews*, *125*, 493–502. https://doi.org/10.1016/j.neubiorev.2021.03.003

Binder, E. B. (2009). The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology*, *34*, S186–S195. https://doi.org/10.1016/j.psyneuen.2009.05.021

Bleker, L. S., Milgrom, J., Sexton-Oates, A., Roseboom, T. J., Gemmill, A. W., Holt, C. J., Saffery, R., Burger, H., & de Rooij, S. R. (2019). Exploring the effect of antenatal depression treatment on children's epigenetic profiles: Findings from a pilot randomized controlled trial. *Clinical Epigenetics*, *11*(1), 18. https://doi.org/10.1186/s13148-019-0616-2

Braithwaite, E. C., Cole, J., Murgatroyd, C., Wright, N., O'Farrelly, C., Barker, B., & Ramchandani, P. (2023). Child DNA methylation in a randomised controlled trial of a video-feedback intervention to promote positive parenting and sensitive discipline (VIPP-SD). *Frontiers in Child and Adolescent Psychiatry*, *2*, 1175299. https://doi.org/10.3389/frcha.2023.1175299

Braun, P. R., Han, S., Hing, B., Nagahama, Y., Gaul, L. N., Heinzman, J. T., Grossbach, A. J., Close, L., Dlouhy, B. J., Howard, M. A., Kawasaki, H., Potash, J. B., & Shinozaki, G. (2019). Genome-wide DNA methylation comparison between live human brain and peripheral tissues within individuals. *Translational Psychiatry*, *9*(1), 47. https://doi.org/10.1038/s41398-019-0376-y

Brody, G. H., Yu, T., Chen, E., Beach, S. R. H., & Miller, G. E. (2016). Family-centered prevention ameliorates the longitudinal association between risky family processes and epigenetic aging. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *57*(5), 566–574. https://doi.org/10.1111/jcpp.12495

Byrne, M. L., Horne, S., O'Brien-Simpson, N. M., Walsh, K. A., Reynolds, E. C., Schwartz, O. S., Whittle, S., Simmons, J. G., Sheeber, L., & Allen, N. B. (2017). Associations between observed parenting behavior and adolescent inflammation two and a half years later in a community sample. *Health Psychology*, *36*(7), 641–651. https://doi.org/10.1037/hea0000502

Cecil, C. A. M., Zhang, Y., & Nolte, T. (2020). Childhood maltreatment and DNA methylation: A systematic review. *Neuroscience & Biobehavioral Reviews*, *112*, 392–409. https://doi.org/10.1016/j.neubiorev.2020.02.019

Chhangur, R. R., Weeland, J., Overbeek, G., Matthys, W., & Orobio De Castro, B. (2012). ORCHIDS: An observational randomized controlled trial on childhood differential susceptibility. *BMC Public Health*, *12*(1), 917. https://doi.org/10.1186/1471-2458-12-917

Conradt, E., Dylan Guerin, D. A., Marsit, C. J., Hawes, K., & Tronick, E. (2016). The contributions of maternal sensitivity and maternal depressive symptoms to epigenetic processes and neuroendocrine functioning. *Child Development*, *87*(1), 73–85. https://doi.org/10.1111/cdev.12483

Conradt, E., Ostlund, B., Guerin, D., Armstrong, D. A., Marsit, C. J., Tronick, E., LaGasse, L., & Lester, B. M. (2019). DNA methylation of NR3C1 in infancy: Associations between maternal caregiving and infant sex. *Infant Mental Health Journal*, *40*(4), 513–522. https://doi.org/10.1002/imhj.21789

Creasey, N., Beijers, R., O'Donnell, K. J., de Weerth, C., & Tollenaar, M. S. (2023). Maternal sensitivity and child internalizing and externalizing behavior: A mediating role for glucocorticoid receptor gene (*NR3C1*) methylation? *Development and Psychopathology*, 1–12. https://doi.org/10.1017/S0954579423000226

Dadds, M. R., Moul, C., Hawes, D. J., Diaz, A. M., & Brennan, J. (2015). Individual differences in childhood behavior disorders associated with epigenetic modulation of the cortisol receptor gene. *Child Development*, *86*(5), 1311–1320. https://doi.org/10.1111/cdev.12391

Dall' Aglio, L., Rijlaarsdam, J., Mulder, R. H., Neumann, A., Felix, J. F., Kok, R., Bakermans-Kranenburg, M. J., van Ijzendoorn, M. H., Tiemeier, H., & Cecil, C. A. M. (2020). Epigenome-wide associations between observed maternal sensitivity and offspring DNA methylation: A population-based prospective study in children. *Psychological Medicine*, *52*, 2481–2491. https://doi.org/10.1017/S0033291720004353

Du, P., Zhang, X., Huang, C., Jafari, N., Kibbe, W. A., Hou, L., & Lin, S. M. (2010). Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis. *BMC Bioinformatics*, *11*(1), 587. https://doi.org/10.1186/1471-2105-11-587

Edgar, R. D., Jones, M. J., Robinson, W. P., & Kobor, M. S. (2017). An empirically driven data reduction method on the human 450K methylation array to remove tissue specific non-variable

CpGs. *Clinical Epigenetics*, *9*(1), 11. https://doi.org/10.1186/s13148-017-0320-z

Eyberg, S., & Pincus, D. (1999). Eyberg Child Behavior Inventory and Sutter-Eyberg Student Behavior Inventory-revised: Professional manual. In *Psychological assessment resources*. http://ci.nii.ac.jp/naid/10028059584/en/

Eyberg, S. M., & Robinson, E. A. (1983). Conduct problem behavior: Standardization of a behavioral rating scale with adolescents. *Journal of Clinical Child Psychology*, *12*(3), 347–354.

Flouri, E., Midouhas, E., Ruddy, A., & Moulton, V. (2017). The role of socio-economic disadvantage in the development of comorbid emotional and conduct problems in children with ADHD. *European Child & Adolescent Psychiatry*, *26*(6), 723–732. https://doi.org/10.1007/s00787-017-0940-z

Fortin, J.-P., Labbe, A., Lemire, M., Zanke, B. W., Hudson, T. J., Fertig, E. J., Greenwood, C. M., & Hansen, K. D. (2014). Functional normalization of 450k methylation array data improves replication in large cancer studies. *Genome Biology*, *15*(11), 503. https://doi.org/10.1186/s13059-014-0503-2

Gardini, E. S., Schaub, S., Neuhauser, A., Ramseier, E., Villiger, A., Ehlert, U., Lanfranchi, A., & Turecki, G. (2022). Methylation of the glucocorticoid receptor promoter in children: Links with parents as teachers, early life stress, and behavior problems. *Development and Psychopathology*, *34*, 810–822. https://doi.org/10.1017/S0954579420001984

Gatti, U., Grattagliano, I., & Rocca, G. (2019). Evidence-based psychosocial treatments of conduct problems in children and adolescents: An overview. *Psychiatry, Psychology and Law*, *26*(2), 171–193. https://doi.org/10.1080/13218719.2018.1485523

Gunnar, M. R. (2017). Social buffering of stress in development: A career perspective. *Perspectives on Psychological Science*, *12*(3), 355–373. https://doi.org/10.1177/1745691616680612

Gutman, L. M., Joshi, H., & Schoon, I. (2019). Developmental trajectories of conduct problems and cumulative risk from early childhood to adolescence. *Journal of Youth and Adolescence*, *48*(2), 181–198. https://doi.org/10.1007/s10964-018-0971-x

Hartman, C. A., Hermanns, V. W., de Jong, P. J., & Ormel, J. (2013). Self- or parent report of (co-occurring) internalizing and externalizing problems, and basal or reactivity measures of HPA-axis functioning: A systematic evaluation of the internalizing-hyperresponsivity versus externalizing-hyporesponsivity HPA-axis hypothesis. *Biological Psychology*, *94*(1), 175–184. https://doi.org/10.1016/j.biopsycho.2013.05.009

Heinrich, A., Buchmann, A. F., Zohsel, K., Dukal, H., Frank, J., Treutlein, J., Nieratschker, V., Witt, S. H., Brandeis, D., Schmidt, M. H., Esser, G., Banaschewski, T., Laucht, M., & Rietschel, M. (2015). Alterations of glucocorticoid receptor gene methylation in externalizing disorders during childhood and adolescence. *Behavior Genetics*, *45*(5), 529–536. https://doi.org/10.1007/s10519-015-9721-y

Hoye, J. R., Cheishvili, D., Yarger, H. A., Roth, T. L., Szyf, M., & Dozier, M. (2019). Preliminary indications that the attachment and biobehavioral catch-up intervention alters DNA methylation in maltreated children. *Development and Psychopathology*, *1–9*, 1486–1494. https://doi.org/10.1017/S0954579419001421

Jones, M., Moore, S., & Kobor, M. (2018). Principles and challenges of applying epigenetic epidemiology to psychology. *Annual Review of Psychology*, *69*, 459–485. https://doi.org/10.1146/annurev-psych-122414-033653

Kim-Cohen, J., Caspi, A., Moffitt, T. E., Harrington, H., Milne, B. J., & Poulton, R. (2003). Prior juvenile diagnoses in adults with mental disorder: Developmental follow-back of a prospective-longitudinal cohort. *Archives of General Psychiatry*, *60*(7), 709–717. https://doi.org/10.1001/archpsyc.60.7.709

Klengel, T., Divya, M., Anacker, C., Rex-Haffner, M., Pruessner, J. C., Pariante, C., Pace, T. W. W., Mercer, K. B., Mayberg, H. S., Bradley, B., Nemeroff, C. B., Holsboer, F., Heim, C. M., Ressler, K. J., Rein, T., & Binder, E. B. (2013). Allele-specific FKBP5 DNA demethylation mediates gene–childhood trauma interactions. *Nature Neuroscience*, *16*(1), 33–41. https://doi.org/10.1038/nn.3275

Laurent, H. K., Harold, G. T., Leve, L., Shelton, K. H., & Van Goozen, S. H. M. (2016). Understanding the unfolding of stress regulation in infants. *Development and Psychopathology*, *28*(4pt2), 1431–1440. https://doi.org/10.1017/S0954579416000171

Leijten, P., Gardner, F., Landau, S., Harris, V., Mann, J., Hutchings, J., Beecham, J., Bonin, E. M., & Scott, S. (2018). Research review: Harnessing the power of individual participant data in a meta-analysis of the benefits and harms of the Incredible Years parenting program. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *59*(2), 99–109. https://doi.org/10.1111/jcpp.12781

Li, M., Zou, D., Li, Z., Gao, R., Sang, J., Zhang, Y., Li, R., Xia, L., Zhang, T., Niu, G., Bao, Y., & Zhang, Z. (2019). EWAS Atlas: A curated knowledgebase of epigenome-wide association studies. *Nucleic Acids Research*, *47*(D1), D983–D988. https://doi.org/10.1093/nar/gky1027

Marsman, R., Swinkels, S. H. N., Rosmalen, J. G. M., Oldehinkel, A. J., Ormel, J., & Buitelaar, J. K. (2008). HPA-axis activity and externalizing behavior problems in early adolescents from the general population: The role of comorbidity and gender. *Psychoneuroendocrinology*, *33*(6), 789–798. https://doi.org/10.1016/j.psyneuen.2008.03.005

McCoy, E. (2017). Understanding the intention-to-treat principle in randomized controlled trials. *The Western Journal of Emergency Medicine*, *18*(6), 1075–1078. https://doi.org/10.5811/westjem.2017.8.35985

McEwen, B. S., & Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior*, *43*(1), 2–15. https://doi.org/10.1016/S0018-506X(02)00024-7

Menting, A. T. A., Orobio De Castro, B., & Matthys, W. (2013). Effectiveness of the Incredible Years parent training to modify disruptive and prosocial child behavior: A meta-analytic review. *Clinical Psychology Review*, *33*(8), 901–913. https://doi.org/10.1016/j.cpr.2013.07.006

Mi, H., Ebert, D., Muruganujan, A., Mills, C., Albou, L., & Mushayamaha, T. (2020). PANTHER version 16: a revised family classification, tree-based classification tool, enhancer regions and extensive API. *Nucleic Acids Research*, *49*(D1), D394–D403. https://doi.org/10.1093/nar/gkaa1106

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

Nelles-McGee, T., Khoury, J., Kenny, M., Joshi, D., & Gonzalez, A. (2022). Biological embedding of child maltreatment: A systematic review of biomarkers and resilience in children and youth. *Psychological Trauma Theory Research Practice and Policy*, *14*(S1), S50–S62. https://doi.org/10.1037/tra0001162

O'Donnell, K. J., Chen, L., MacIsaac, J. L., McEwen, L. M., Nguyen, T., Beckmann, K., Zhu, Y., Chen, L. M., Brooks-Gunn, J., Goldman, D., Grigorenko, E. L., Leckman, J. F., Diorio, J., Karnani, N., Olds, D. L., Holbrook, J. D., Kobor, M. S., & Meaney, M. J. (2018). DNA methylome variation in a perinatal nurse-visitation program that reduces child maltreatment: A 27-year follow-up. *Translational Psychiatry*, *8*(1), 15. https://doi.org/10.1038/s41398-017-0063-9

Oliveira, P., & Fearon, P. (2019). The biological bases of attachment. *Adoption and Fostering*, *43*(3), 274–293. https://doi.org/10.1177/0308575919867770

Ong, M.-L., Lin, X., & Holbrook, J. D. (2015). Measuring epigenetics as the mediator of gene/environment interactions in DOHaD. *Journal of Developmental Origins of Health and Disease*, *6*(1), 10–16. https://doi.org/10.1017/S2040174414000506

Overbeek, G., van Aar, J., de Castro, B. O., Matthys, W., Weeland, J., Chhangur, R. R., & Leijten, P. (2020). Longer-term outcomes of

the Incredible Years parenting intervention. *Prevention Science*, *22*, 419–431. https://doi.org/10.1007/s11121-020-01176-6

Parade, S. H., Huffhines, L., Daniels, T. E., Stroud, L. R., Nugent, N. R., & Tyrka, A. R. (2021). A systematic review of childhood maltreatment and DNA methylation: Candidate gene and epigenome-wide approaches. *Translational Psychiatry*, *11*(1), 134. https://doi.org/10.1038/s41398-021-01207-y

Patterson, G. R. (2016). Coercion theory: The study of change. In T. J. Dishion & J. J. Snyder (Eds.), *The Oxford handbook of coercive relationship dynamics* (pp. 7–22). Oxford University Press.

Phipson, B., Maksimovic, J., & Oshlack, A. (2016). missMethyl: An R package for analyzing data from Illumina's HumanMethylation450 platform. *Bioinformatics*, *32*(2), 286–288. https://doi.org/10.1093/bioinformatics/btv560

Provençal, N., Arloth, J., Cattaneo, A., Anacker, C., Cattane, N., Wiechmann, T., Röh, S., Ködel, M., Klengel, T., Czamara, D., Müller, N. S., Lahti, J., Räikkönen, K., Pariante, C. M., Binder, E. B., Kajantie, E., Hämäläinen, E., Villa, P., & Laivuori, H. (2020). Glucocorticoid exposure during hippocampal neurogenesis primes future stress response by inducing changes in DNA methylation. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(38), 23280–23285. https://doi.org/10.1073/pnas.1820842116

Provenzi, L., Brambilla, M., Scotto di Minico, G., Montirosso, R., & Borgatti, R. (2020). Maternal caregiving and DNA methylation in human infants and children: Systematic review. *Genes, Brain and Behavior*, *19*: 1–11. https://doi.org/10.1111/gbb.12616

Rich, B. A., & Eyberg, S. M. (2001). Accuracy of assessment: The discriminative and predictive power of the Eyberg Child Behavior Inventory. *Ambulatory Child Health*, *7*(3–4), 249–257. https://doi.org/10.1046/j.1467-0658.2001.00141.x

Robinson, E. A., & Eyberg, S. M. (1981). The dyadic parent–child interaction coding system: Standardization and validation. *Journal of Consulting and Clinical Psychology*, *49*(2), 245–250. https://doi.org/10.1037/0022-006X.49.2.245

Ruttle, P. L., Shirtcliff, E. A., Serbin, L. A., Ben-Dat Fisher, D., Stack, D. M., & Schwartzman, A. E. (2011). Disentangling psychobiological mechanisms underlying internalizing and externalizing behaviors in youth: Longitudinal and concurrent associations with cortisol. *Hormones and Behavior*, *59*(1), 123–132. https://doi.org/10.1016/j.yhbeh.2010.10.015

Shanthikumar, S., Neeland, M. R., Maksimovic, J., Ranganathan, S. C., & Saffery, R. (2020). DNA methylation biomarkers of future health outcomes in children. *Molecular and Cellular Pediatrics*, *7*(1), 7. https://doi.org/10.1186/s40348-020-00099-0

Shirtcliff, E. A., Skinner, M. L., Obasi, E. M., & Haggerty, K. P. (2017). Positive parenting predicts cortisol functioning six years later in young adults. *Developmental Science*, *20*(6), e12461. https://doi.org/10.1111/desc.12461

Sjöstedt, E., Zhong, W., Fagerberg, L., Karlsson, M., Mitsios, N., Adori, C., Oksvold, P., Edfors, F., Limiszewska, A., Hikmet, F., Huang, J., Du, Y., Lin, L., Dong, Z., Yang, L., Liu, X., Jiang, H., Xu, X., Wang, J., … Mulder, J. (2020). An atlas of the protein-coding genes in the human, pig, and mouse brain. *Science*, *367*(6482), eaay5947. https://doi.org/10.1126/science.aay5947

Smeekens, S., Marianne Riksen-Walraven, J., & van Bakel, H. J. A. (2007). Cortisol reactions in five-year-olds to parent–child interaction: The moderating role of ego-resiliency. *Journal of Child Psychology and Psychiatry*, *48*(7), 649–656. https://doi.org/10.1111/j.1469-7610.2007.01753.x

Starnawska, A., & Demontis, D. (2021). Role of DNA methylation in mediating genetic risk of psychiatric disorders. *Frontiers in Psychiatry*. https://doi.org/10.3389/fpsyt.2021.596821

Suderman, M., Staley, J. R., French, R., Arathimos, R., Simpkin, A., & Tilling, K. (2018). Dmrff: Identifying differentially methylated regions efficiently with power and control. *bioRxiv*. https://doi.org/10.1101/508556

Szyf, M., & Bick, J. (2013). DNA methylation: A mechanism for embedding early life experiences in the genome. *Child Development*, *84*(1), 49–57. https://doi.org/10.1111/j.1467-8624.2012.01793.x

Szyf, M., McGowan, P., & Meaney, M. J. (2008). The social environment and the epigenome. *Environmental and Molecular Mutagenesis*, *49*(1), 46–60. https://doi.org/10.1002/em.20357

Teschendorff, A. E., Zhuang, J., & Widschwendter, M. (2011). Independent surrogate variable analysis to deconvolve confounding factors in large-scale microarray profiling studies. *Bioinformatics*, *27*(11), 1496–1505. https://doi.org/10.1093/bioinformatics/btr171

Tyrka, A. R., Ridout, K. K., & Parade, S. H. (2016). Childhood adversity and epigenetic regulation of glucocorticoid signaling genes: Associations in children and adults. *Development and Psychopathology*, *28*(4), 1319–1331. https://doi.org/10.1017/S0954579416000870

Uhlén, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, Å., Kampf, C., Sjöstedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C. A.-K., Odeberg, J., Djureinovic, D., Takanen, J. O., Hober, S., … Pontén, F. (2015). Tissue-based map of the human proteome. *Science*, *347*(6220), 1260419. https://doi.org/10.1126/science.1260419

Wadji, D. L., Tandon, T., Ketcha Wanda, G. J. M., Wicky, C., Dentz, A., Hasler, G., Morina, N., & Martin-Soelch, C. (2021). Child maltreatment and NR3C1 exon 1F methylation, link with deregulated hypothalamus-pituitary-adrenal axis and psychopathology: A systematic review. *Child Abuse & Neglect*, *122*(September), 105304. https://doi.org/10.1016/j.chiabu.2021.105304

Weaver, I. C. G. (2007). Epigenetic programming by maternal behavior and pharmacological intervention nature versus nurture: Let's call the whole thing off. *Epigenetics*, *2*(1), 22–28. https://doi.org/10.4161/epi.2.1.3881

Weaver, I. C. G. (2009). Epigenetic effects of glucocorticoids. *Seminars in Fetal & Neonatal Medicine*, *14*(3), 143–150. https://doi.org/10.1016/j.siny.2008.12.002

Weaver, I. C. G., Champagne, F. A., Brown, S. E., Dymov, S., Sharma, S., Meaney, M. J., & Szyf, M. (2005). Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: Altering epigenetic marking later in life. *The Journal of Neuroscience*, *25*(47), 11045–11054. https://doi.org/10.1523/JNEUROSCI.3652-05.2005

Weaver, I. C. G., D'Alessio, A. C., Brown, S. E., Hellstrom, I. C., Dymov, S., Sharma, S., Szyf, M., & Meaney, M. J. (2007). The transcription factor nerve growth factor-inducible protein a mediates epigenetic programming: Altering epigenetic Marks by immediate-early genes. *Journal of Neuroscience*, *27*(7), 1756–1768. https://doi.org/10.1523/JNEUROSCI.4164-06.2007

Webster-Stratton, C. (2014). The Incredible Years: Parents, teachers, and children training series. In *Innovative Mental Health Interventions for Children: Programs that Work* (pp. 31–45). Routledge. https://doi.org/10.4324/9781315786179

Webster-Stratton, C., Reid, M. J., & Hammond, M. A. (2001). Preventing conduct problems, Promoting Social Competence: A parent and teacher training partnership in head start. *Journal of Clinical Child and Adolescent Psychology*, *30*(3), 283–302. https://doi.org/10.1207/s15374424jccp3003_2

Weeland, J., Aar, J. V., & Overbeek, G. (2018). Dutch norms for the Eyberg Child Behavior Inventory: Comparisons with other Western countries. *Journal of Psychopathology and Behavioral Assessment*, *40*, 224–234.

Weeland, J., Chhangur, R. R., van der Giessen, D., Matthys, W., de Castro, B. O., & Overbeek, G. (2017). Intervention effectiveness of the Incredible Years: New insights into sociodemographic and intervention-based moderators. *Behavior Therapy*, *48*(1), 1–18. https://doi.org/10.1016/j.beth.2016.08.002

Wesarg, C., Van Den Akker, A. L., Oei, N. Y. L., Hoeve, M., & Wiers, R. W. (2020). Identifying pathways from early adversity to psychopathology: A review on dysregulated HPA axis functioning

and impaired self-regulation in early childhood. *European Journal of Developmental Psychology*, *17*(6), 808–827. https://doi.org/10.1080/17405629.2020.1748594

Wheater, E., Galdi, P., McCartney, D. L., Blesa, M., Sullivan, G., Stoye, D. Q., Lamb, G. J., Sparrow, S., Murphy, L., Wrobel, N., Quigley, A. J., Semple, S., Thrippleton, M. J., Wardlaw, J. M., Bastin, M. E., Marioni, R. E., Cox, S. R., & Boardman, J. P. (2022). DNA methylation in relation to gestational age and brain dysmaturation in preterm infants. *Brain Communications*, *4*(2). https://doi.org/10.1093/braincomms/fcac056

Wiechmann, T., Röh, S., Sauer, S., Czamara, D., Arloth, J., Ködel, M., Beintner, M., Knop, L., Menke, A., Binder, E. B., & Provençal, N. (2019). Identification of dynamic glucocorticoid-induced methylation changes at the FKBP5 locus. *Clinical Epigenetics*, *11*(1), 83. https://doi.org/10.1186/s13148-019-0682-5

Yuan, N., Chen, Y., Xia, Y., Dai, J., & Liu, C. (2019). Inflammation-related biomarkers in major psychiatric disorders: A cross-disorder assessment of reproducibility and specificity in 43 meta-analyses. *Translational Psychiatry*, *9*(1), 233. https://doi.org/10.1038/s41398-019-0570-y

Zannas, A. S., Wiechmann, T., Gassen, N. C., & Binder, E. B. (2016). Gene–stress–epigenetic regulation of FKBP5: Clinical and translational implications. *Neuropsychopharmacology*, *41*(1), 261–274. https://doi.org/10.1038/npp.2015.235

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