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Epigenetic Patterns Modulate the Connection Between Developmental Dynamics of Parenting and Offspring Psychosocial Adjustment

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This study attempted to establish and quantify the connections between parenting, offspring psychosocial adjustment, and the epigenome. The participants, 35 African American young adults (19 females and 16 males; age = 17–29.5 years), represented a subsample of a 3-wave longitudinal 15-year study on the developmental trajectories of low-income urban mother–offspring dyads. Mothers were assessed on their perceptions of maternal stress at each wave. Offspring were assessed on their perceptions of maternal parenting at each wave and on their adaptive and maladaptive behavior at the last wave. Genome-wide DNA methylation in peripheral T lymphocytes at the third wave was assayed using Methyl Binding Domain(MBD) sequencing. Statistically significant associations were identified between the change in offspring's perception of parenting from middle childhood to adulthood and the DNA methylation in offspring's adult genomes. Specifically, the slope of perceived parental rejection across the 3 time points was related to an increase in methylation, or a potential downregulation, of 565 genes thought to be involved in the control of a broad spectrum of biological functions generally related to cellular signaling. A subset of these epigenetic marks, clustered in 23 genes, some of which participate in the development and functioning of the CNS, were in turn associated with psychosocial adjustment as captured by interpersonal relationships and emotional self-evaluation. This appears to be one of the first investigations of the modulating role of the methylome in associations between developmental dynamics of parenting throughout the formative years of child and adolescent development and psychosocial adjustment in adulthood.

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There is mounting evidence indicating that the relations between parenting and child behavior, emotional well-being, and psychopathology are reciprocal over time (Pardini, 2008). Parenting behavior is subject to short-term and long-term temporal

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dynamics, with a potential cascading effect on children's psychosocial adjustment later in life exerted not only by type, but also by the stability of the patterns of parent–child interactions (Cox, Mills-Koonce, Propper, & Gariepy, 2010). Despite the growing consensus that both parenting and its life-span fluctuations impact children's development, the complex multilayered mechanisms behind these transactional effects are yet to be fully understood (Barbot, Crossman, Hunter, Grigorenko, & Luthar, 2014; Pettit & Arsiwalla, 2008). Parents' psychological well-being, characteristics of parenting, and children's perception of the parent–child relationship are interrelated, hampering inferences about the directionality of effects or the disentangling of the role of each of these variables in predicting children's adjustment (Patterson & Fisher, 2002).

A number of factors have been proposed as potential modulators (or *modulating factors*) that include mediation, moderation, and all other types of nontrivial associations between two variables of interest in the presence of a third variable, in the relation between parenting and child adjustment. At the societal level such factors include the amount and quality of child care (Romano, Kohen, & Findlay, 2010). At the neurobiological level such factors include low stress reactivity or optimized prefrontal cortex functioning (Bloomfield, 2011; Rutter, 2012; Silk et al., 2007). In addition, it is acknowledged that parental stress and mental health impact both children's brain and behavior development (Dawson, Ashman, & Carver, 2000), with potential long-term effects on adjustment (Scott, 2012). Despite these and other relevant observations, a framework for understanding the biological underpinnings linking parenting and children's experiences of parent–child relationships with their long-term outcomes on adjustment has not been developed.

During the last decade, epigenetic mechanisms (e.g., histone modifications and DNA methylation) have become central to studies of the modulation between the dynamics of relevant environments and specific phenotypes. Epigenetic mechanisms connecting social environment and behavioral phenotypes are studied within the behavioral epigenetics field (Lester et al., 2011). The basic premises set by this field imply that social environments impact the epigenome in a lasting way, generating persistent and system-wide changes in gene function (McGowan et al., 2009; Suderman et al., 2014; Szyf, 2013; Szyf, McGowan, & Meaney, 2008; Weaver et al., 2004) that, in turn, change relevant physiology (e.g., neuronal plasticity and functioning) and, consequently, affect behavior. However, we do not have a detailed picture of the affected regions of the epigenome and the pathways through which they modulate behavior. This is mainly due to the field's still emerging understanding of (a) the specificities of epigenetic regulation (e.g., DNA methylation) and its role in social experiences, and (b) the specificities of social experiences (e.g., typologies of negative life events) as contexts for epigenome reactivity.

Here, we offer a novel answer to the "old question" regarding what mechanisms modulate the effects of parenting on offspring's long-term adjustment. Our study, grounded in the epigenetic literature, investigates whether epigenetic mechanisms are a candidate for forwarding the effects of parenting to offspring outcomes in adulthood. Specifically, this article reports on associations between parenting behavior, namely, offspring's perceived parenting and mothers' perceived parenting stress and the offspring's epigenetic patterns (DNA methylation marks), and the associations of these epigenetic marks with psychosocial adjustment in adulthood. Due to a shortage of knowledge on dynamic associations between the epigenome and social experiences during the human life span, it is difficult to formulate specific hypotheses. Yet, capitalizing on what is known about the dynamics of parenting and corresponding reflections on the epigenome (Weaver et al., 2004), we hypothesized that indicators of parenting across three distinct developmental periods—middle childhood, adolescence, and young adulthood—might be more sensitive to capturing an association with the changes in the methylome as they would differentiate stable and variable characteristics of parenting. Given the important role of the stability, not only the type, of the patterns of parenting and mother–child interactions in offspring's psychosocial adjustment, we investigated the association between the epigenome and the temporal dynamics of parenting over these three periods. Specifically, we investigated whether the stability in comparison to variability in parenting (i.e., the perceived change in parenting over time) may have a different degree of influence on the epigenome.

Method

Participants

The participants in this study were a subsample (35 offspring) of a sample including 361 low-income ethnically diverse urban mother–offspring dyads (see Appendix S1 and Table S1). Mother–offspring

dyads were assessed three times over the course of 15 years following the first measurement occasion (T1) in 1996, with the second (T2) and third (T3) occasions following at approximately 5-year intervals.

A cohort of 35 offspring of African American descent (19 females; age range = 17 to 29.5 years, $M = 22.5 \pm 3.3$ years) was selected to constrain ethnic diversity, contingent upon participants' willingness to donate biospecimens at T3. All participants provided written consent and a blood sample; ethical approval for the study was obtained from the proper authorities of the institutions involved in this study. Age, gender, and the presence of substance-related and addictive disorders (SRAD) and/or psychiatric problems of the participants were used as covariates in the analyses. Additional demographic information is provided in Appendix S1.

Indicators of Parenting

Detailed information on the measures used to assess the indicators of parenting is included in Appendix S1. The Parental Acceptance–Rejection Questionnaire (PARQ; Khaleque & Rohner, 2002) was used to assess offsprings' perceptions of their relationships with mothers. This self-report questionnaire has four subscales (warmth and affection; hostility and aggression; indifference and neglect; and undifferentiated rejection) and comprises 60 items rated on a 4-point scale from 0 (*never true of my mother*) to 3 (*almost always true of my mother*).

Mothers rated their experiences of parenting stress using the Parenting Stress Index–Short Form (PSI–SF; Abidin, 1995), a 36-item self-report measure that uses a 5-point Likert scale (ranging from 1 = *strongly disagree* to 5 = *strongly agree*). The measure yields a total stress score from three subscales with 12 items each, including (a) parental distress, (b) parent–child dysfunctional interactions, and (c) the difficult child subscale.

Two indicators were calculated at each of the three time points: (a) indicators of perceived parental rejection computed as the sum of the hostility–aggression, indifference–neglect, and undifferentiated rejection subscales scores of the PARQ and (b) indicators of maternal stress computed as the sum score of the three aforementioned PSI subscale scores. Finally, scores across all time points were averaged to gauge the general level of perceived parental rejection (Cronbach's $\alpha = .60$) and maternal stress (Cronbach's $\alpha = .90$) across the entire study period (see Table S2).

Indicators of Temporal Dynamics of Parenting

Beyond concurrent individual differences in the perception of parental rejection, some children may cope differently with the experience of rejection during different developmental periods. Therefore, we focused on investigating the influences of perceived parental rejection over time (T1–T3) on current epigenetic patterns (T3). Two linear latent growth curve models (Preacher, Wichman, MacCallum, & Briggs, 2008) were specified to derive slope values of parental rejection and maternal stress for subsequent epigenome-wide association study (EWAS) analyses, using a latent variable comprising observed PARQ and PSI subscale scores as indicators for each time point, respectively. This growth curve modeling approach is described in detail in Appendix S1. The factor scores of the slopes were computed (regression method using posterior means) and used as indicators of linear change in parenting, based on perceptions of parental rejection and maternal stress (PARQ slope and PSI slope, respectively; see Table S2).

Indicators of Psychosocial Adjustment

Detailed information on the measure used to assess indicators of psychosocial adjustment is included in Appendix S1. The college version of the Behavior Assessment System for Children (BASC–CV; Nowinski, Furlong, Rahban, & Smith, 2007) was used to assess five indicators of psychosocial adjustment: (a) internalizing problems, (b) inattention/hyperactivity, (c) emotional symptoms index, (d) personal adjustment, and (e) a clinical composite. Descriptive statistics on the BASC–CV scores are available in Table S3.

Genome-Wide DNA Methylation Profiling

Detailed information on the approach to genome-wide DNA methylation profiling is included in Appendix S1. To decrease the variability in DNA methylation profiles due to the high cell specificity of DNA methylation patterns and differential white blood cell counts among individuals, we focused on a single cell type, T lymphocytes (CD3). For genome-wide DNA methylation profiling, methyl-CpG binding domain sequencing (MBD-seq) was utilized, which consists of the capture of methylated DNA (ME-DNA) using the MBD domain of MBD2, and subsequent next generation sequencing of the eluted DNA. The Illu-

mina HiSeq platform was used for the massive parallel sequencing of the ME-DNA; paired-end sequencing (2 × 75 bp reads) was utilized. MBD-seq outcomes and the results of the alignment are summarized in Table S4.

Each genome-wide profile was represented by a methylation measurement matrix of around 10 M 300-bp fragments. For the EWAS analyses 480,172 autosomal fragments or markers (ME markers) that contain the most variation in methylation levels and represent approximately 5% of the genome were used. Details on cutoff criteria for establishing the final list of ME markers are given in Appendix S1.

Epigenome-Wide Association Analyses

To examine the associations between parenting and DNA methylation, we used multiple linear regression analyses. We ran separate ordinary least squares regressions to regress the methylation levels of 480,172 selected ME markers on the covariates (Table S1) and one of four parenting indicators, that is, the means and slopes of the PARQ and PSI (Table S2). The goal of this approach was to determine the set of ME markers (thereafter, parenting-associated ME markers) that were significantly associated with a parenting indicator over and above the effects of potentially confounding variables (i.e., age, gender, and the number of SRAD and psychiatric problems). The p value associated with the t test, indicating whether the unstandardized regression coefficient of parenting was significantly different from zero, was adjusted for multiple comparisons using the Benjamini–Hochberg correction (false discovery rate [FDR]; Benjamini & Hochberg, 1995). Coefficients below .05 according to the FDR-adjusted p values were retained as statistically significant. Moreover, residuals of the regression models were evaluated for normality using the Shapiro–Wilk test for the markers after the amendment for multiple comparisons. The models that resulted in non-normal distributions of residuals were considered insufficiently robust and disregarded in subsequent analyses.

We then examined whether the resulting sets of parenting-associated ME markers might be related to psychosocial adjustment. Specifically, linear regression models were fitted using the five BASC–CV composites (Table S3) as dependent variables, methylation levels of parenting-associated ME markers as independent variables, and the aforementioned as covariates.

Gene Annotation of the Methylation Fragments and Functional Analysis of Genes

To relate the sets of parenting-associated ME markers to genes and functional genomic regions, the ME markers were subsequently annotated to the GRCh37/hg19 human genome assembly, using the bedtools software package (https://github.com/arq5x/bedtools2). Each marker was intersected both within a gene and a 2-kb upstream region. To connect the resulting sets of parenting-associated genes to biological functions, as they are defined in Gene Ontology (GO) terms (cellular components, molecular functions, and biological processes), the Database for Annotation, Visualization, and Integrated Discovery (DAVID) was used (http://david.abcc.ncifcrf.gov). To appraise the activity of the genes and the variability of their expression levels in blood tissue, a coexpression network for the genes was constructed via the WebQTL resource (http://www.genenetwork.org), which combines genetic and phenotypic databases using specific analytic tools. Specifically, the data on the mRNA expression in the blood from the Genotype–Tissue Expression (GTEx) project (Lonsdale et al., 2013) were used for the network construction.

Results

The present study conceptualizes epigenetic marks as potential modulators of the relation between parenting and long-term psychosocial adjustment. Results are presented in three sections: (a) for the behavior analyses, the association between perceived parental rejection and adult psychosocial outcomes; (b) for the EWAS analyses, the identification of regions in the methylome that are associated with different indicators of parenting; and (c) for the combined behavior–methylome analyses, the association between the methylome markers identified in (b) and indicators of psychosocial adjustment.

Perceived Parental Rejection Is Associated With Adult Psychosocial Outcomes

As expected, parenting variables were related to offspring psychosocial adjustment. Specifically, the increase in offspring's perception of parental rejection over time (PARQ slope) was related to higher levels (all $ps < .001$) of internalizing problems

(r = .59), inattention/hyperactivity (r = .52), emotional problems (r = .62), and clinical symptoms (r = .55), and lower levels (r = −.48, p < .01) of personal adjustment in adulthood. The correlations between psychosocial adjustment and the average level of perceived parental rejection across all three time points showed lower values but similar magnitudes: internalizing problems (r = .50), inattention/hyperactivity (r = .49), emotional problems (r = .54), clinical symptoms (r = .52), and personal adjustment (r = −.50), all ps < .01. Thus, there were significant associations between perceived parental rejection (mean and slope) and adult outcomes, whereas maternal stress (PSI slope and averaged PSI scores) was not significantly associated with offspring's adult outcomes.

DNA Methylation Is Associated With Changes in Perceived Parental Rejection

The EWAS did not show significant associations between the epigenetic patterns and the indicators of parenting (PARQ and PSI), when PARQ and PSI measurements were considered separately for each of three developmental periods and were averaged across the three time points. Also, there were no significant associations observed between the linear change in maternal stress (PSI slope) and the ME markers. However, there were 818 significant (p < .05, after multiple testing corrections) associations between ME markers and the indicators of linear change in perceived parental rejection, PARQ slope. The resulting ME markers and statistics for the association with the PARQ slope are shown in Table S5. The age, gender, and mental health indicators, used as covariates in the regression models, did not show any significant contribution to the variability in the methylation levels of these 818 ME markers (see Figure S1).

Most of the 818 ME markers (787 or approximately 96%) were positively related to the PARQ slope. Moreover, mean methylation levels of the parenting-associated markers and the PARQ slope yielded positive correlations (Figure 1A and B). Specifically, a 1-unit increase in the PARQ slope was associated with a 0.11-unit increase in mean methylation levels across the 818 ME markers. However, we interpret this effect cautiously because much of the effect was due to three cases, which show slope values of about 5, while most of the 35 individuals clustered in the range of −2 to 2 on the PARQ slope (Figure 1A). While values this high are possible and, perhaps, true to the assumed biological mechanism, they are discrepant with the remaining cluster of values and thus exert a certain degree of leverage on the fit of the regression model. Although we believe that these are legitimate cases, this finding may indicate that the nature of the studied sample is such that variables are often distributed non-normally. Without the three cases in question, adding the PARQ slope to the model still accounted for a significant amount of variance in the mean methylation level over and above the contribution of the covariates, $F(1, 26) = 4.48$, $p = .04$, Cohen's $f^2 = 0.17$. Moreover, a significant association between the PARQ slope and DNA methylation was confirmed even after removing the three outliers (see Figure 1B), supporting the robustness of the association between change in perception of parental rejection and DNA methylation in specific genomic regions. Notably, the 818 ME markers are widely distributed across the entire genome. Gene annotation of these marks indicated that about 70% of them are allied with 565 different genes (see Table S6); they are located within the sequence or the 2-kb region upstream of these genes. Full results of the functional analysis of these 565 genes are presented in Table S7; a summary of this analysis (e.g., GO categories significantly overrepresented among these genes) is provided in Table 1. The GO annotation demonstrated that the list is especially enriched in genes controlling functions such as GTPase activity and its regulation, ATP binding, and metal ion binding. These molecular functions are involved in a broad spectrum of biological processes and pathways, such as development, the cell cycle, and metabolic processes.

The Epigenetic Links Between Parenting and Psychosocial Adjustment

After multiple testing corrections, 38 of the 818 ME markers showed significant (p < .05) and negative relations with the BASC–CV personal adjustment composite (Table S5). Specifically, lower levels in personal adjustment were associated with higher methylation levels while controlling for age, gender, and the number of SRAD and psychiatric problems. In a model with the mean level of methylation among the 38 identified markers as an independent variable in the regression (Figure 2), the results showed that DNA methylation was significantly and negatively related to the BASC–CV personal adjustment composite (B = −26.80, SE = 7.58, β = −.74, p = .002), but the association between the PARQ slope and BASC–CV personal adjustment composite was not significant over and above the

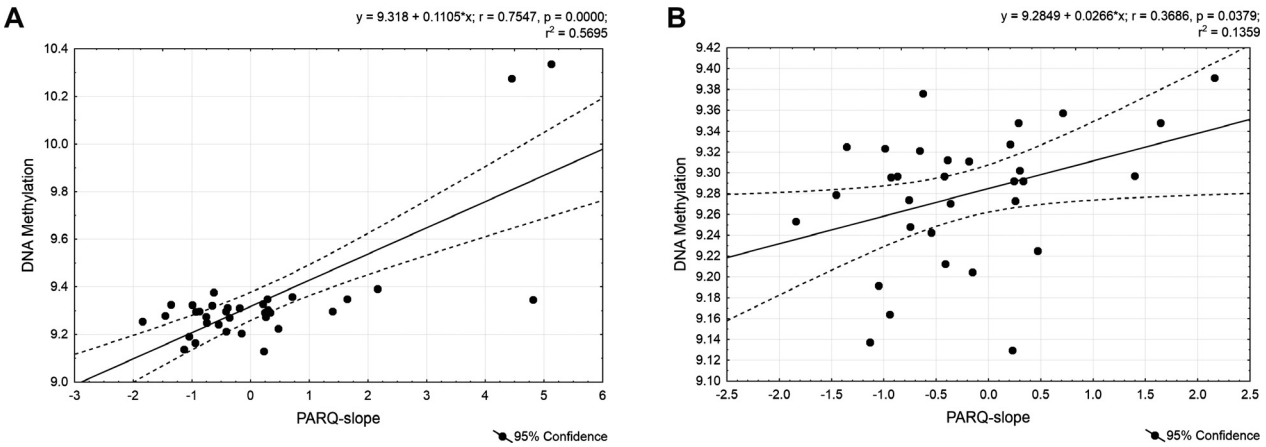

Figure 1. Linear regression model that relates the score of change in perceived parental rejection (Parental Acceptance–Rejection Questionnaire [PARQ] slope) and the mean methylation levels of 818 parenting-associated markers, or 300 bp MBD-seq fragments for the entire sample of 35 individuals (A) and for 32 samples, with the exclusion of extreme cases (B). Panel A shows that the sample contains three extreme cases. One case had an unusually high PARQ slope value (4.82) with average DNA methylation compared to the rest of the sample (i.e., exerting a leverage on the regression line). Two cases showed discrepancies as reflected in high PARQ slope values (5.12 and 4.45) and high DNA methylation. A set of diagnostic analyses of the regression of mean methylation on PARQ slope values and the covariates were performed to identify any concerns with these extreme cases. Results showed (a) no concerning leverage of mean methylation on the fitted values as indicated by hat values (values were 0.48, 0.46, and 0.46; mean hat value was 0.17); (b) no unusual large or small residuals as indicated by most studentized residuals within the $\pm$ 2 range (largest studentized residual was 2.64 with a Bonferroni-adjusted p value of .47); and (c) no major influence on the regression coefficients of PARQ slope as indicated by a plot of residuals against leverage (Cook's d values were 0.87, 0.54, and 0.84). Given the small sample size, we have tested and evaluated other types of robust regression models using high breakdown point (M- or MM-) estimators as these models tend to be less vulnerable to unusual data. Estimators such as "least trimmed squares" and "least median of squares" are alternatives to ordinary least squares regression that account better for the effect of extreme values. However, these methods led to problems of convergence in many of the regression models we tested. In robust regression using M-estimators, Wald-type inference of the significance of coefficients typically requires larger samples—due to the unreliable asymptotic covariance matrix in small samples—than the data available in this study. Thus, although we were aware of the potential influence of these cases on the regression, we decided to utilize ordinary least squares regression but cautiously interpret the results. Panel B shows that the association between the PARQ slope and the mean of ME markers is high and significant within the sample after removing the three extreme cases. The y-axis (mean DNA methylation) has been rescaled to better illustrate the linear interrelation.

other variables in the model (B = 0.15, SE = 2.07, β = .02, p = .943).

Although no other associations with the BASC–CV composite or individual subscales survived corrections for multiple testing, we found suggestive associations prior to p-value corrections. Indicators of social stress, anxiety, and hyperactivity were related to methylation levels for 31 ME markers (with average uncorrected p values across all ME markers of .003, .003, and .006, respectively). Indicators of depression and attention problems were associated with 443 and 337 ME markers (with average uncorrected p values across all 443 and 337 ME markers of 1.33e-04 and 4.85e-04), respectively. The lowest set of FDR-adjusted p values was .07 and .08 for depression and attention problems, respectively.

As per the genome annotation analysis, of the 38 ME markers associated with both the PARQ slope and BASC–CV personal adjustment, 11 were annotated within intergenic regions with unknown regulatory functions, 4 markers were localized within transcription factor binding sites, and the rest of the markers were related to 23 genes (Table 2). The analysis of the mRNA expression of these genes in blood tissue (based on data from the GTEx project; http://www.gtexportal.org) indicated high coexpression (i.e., covariability in their expression levels) across individuals (Figure 3). This observation suggests system-level functionality that might arise from coregulatory and/or cofunctional relations between these genes. The annotation of these genes' biological functions within the GO categories pointed out that almost half (10 of 23) of them are directly involved in the control of neuronal development and CNS functioning (Table 2).

To investigate the modulating role of the 38 ME markers in the relation between the change in parental rejection and psychosocial adjustment, a series of 38 separate regressions of the BASC–CV composite on each of the ME markers and the

Table 1
Summary of the Functional Annotation of 565 Parenting-Associated Genes Performed Using the DAVID Annotation Tool

Function	GO term	Genes count	Genes %	*p* value	Fold enrichment	FDR-adjusted *p* value[a]
GTPase regulator activity	GO:0030695~GTPase regulator activity	26	6.30	3.68E-06	2.89	.0018
	GO:0060589~nucleoside triphosphatase regulator activity	26	6.30	5.44E-06	2.82	.0014
	GO:0005083~small GTPase regulator activity	16	3.87	0.001195	2.62	.0488
ATP binding	GO:0032559~adenyl ribonucleotide binding	57	13.80	5.44E-05	1.71	.0091
	GO:0005524~ATP binding	55	13.32	1.39E-04	1.67	.0139
	GO:0030554~adenyl nucleotide binding	58	14.04	1.19E-04	1.65	.0148
	GO:0001883~purine nucleoside binding	58	14.04	1.78E-04	1.62	.0148
	GO:0001882~nucleoside binding	58	14.04	2.13E-04	1.62	.0152
Metal ion binding	GO:0046872~metal ion binding	119	28.81	6.38E-04	1.29	.0392
	GO:0043169~cation binding	119	28.81	9.40E-04	1.28	.0461
	GO:0005509~calcium ion binding	36	8.72	0.001177	1.76	.0503
Associated with the long qt syndrome[b]		5	1.21	5.74E-05	21.48	.0066

Note. GO = Gene Ontology.
[a]The FDR-adjusted *p* values (≤ .05) were used as inclusion criteria to trim the overrepresented term list. Fold enrichment of each gene group is estimated in comparison to the base set of genes in the Database for Annotation, Visualization, and Integrated Discovery (DAVID) databases (19,235 genes).
[b]The long QT syndrome—a rare inherited heart condition related to the violation of ventricular fibrillation. The enrichment in this functional group of genes (5 of 12 known associates including two candidate genes, *KCNQ1* and *KCNH2*) is considered an unexpected finding. We cannot speculate about the causal association between these genes and parenting. Most likely, the association was obtained because of the presence of the disease in the study cohort, known to be a population with a high risk and frequency of cardiovascular disorders (an assumption we could not confirm due to a lack of medical records), and a possible causal relation between the syndrome and abnormal methylation of the candidate genes. This might be an issue for future investigation of the candidate genes for the disorder.

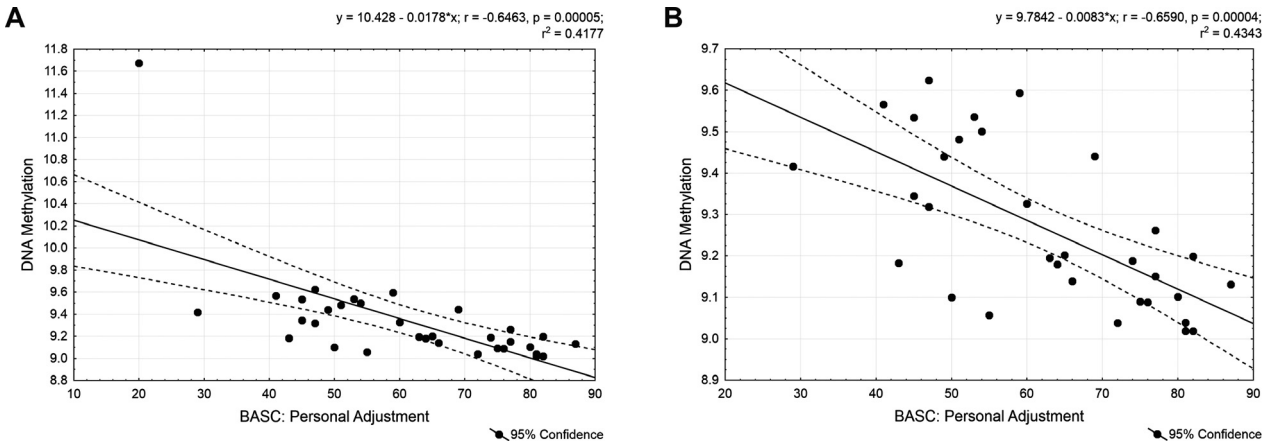

Figure 2. Linear regression model that describes the relation between the Personal Adjustment composite score and the average methylation level of 38 methylation markers that were associated with both the Personal Adjustment and the Parental Acceptance–Rejection Questionnaire slope. Relation were established for the entire studied sample (A) and for the sample without any cases with extremely high methylation levels (B). Both plots show high negative associations between the variables.

PARQ slope were conducted, controlling for age, gender, and the number of SRAD and psychiatric problems. The results showed that 33 of the 38 ME markers related to 20 genes were significantly associated with the BASC–CV personal adjustment over and above the effect of the covariates (Table 2).

Discussion

Although the epigenetic literature has provided evidence for the presence of significant associations between negative early experiences and long-lasting alterations in epigenetic patterns (Borghol et al., 2012; McGowan et al., 2009; Naumova et al., 2012;

Table 2
List of 38 ME Markers That Are Significantly Associated With Both the Linear Change in Perceived Parental Rejection (PARQ Slope) and Psychosocial Adjustment (BASC–CV, Personal Adjustment Composite)

ME marker, start position	Regression coefficients		Genome annotation	
	β	*p* value	Gene/locus	GO: biological process
chr1:33998550	−.461	.039	**CSMD2**	Integral to membrane
chr1:113202150	−.513	*.080*	CAPZA1	Cell motion, cytoskeleton organization, actin filament-based process
chr1:203954250	−.473	.039	TF binding site	
chr2:4183550	−.502	.030		
chr2:11147750	−.555	.030		
chr2:54325850	−.544	.030	ACYP2	Phosphate-containing compound metabolic process
chr2:125652050	−.518	*.056*	**CNTNAP5**	Cell adhesion
chr3:183525750	−.676	.030	YEATS2-AS1	
chr3:196318950	−.634	.035		
chr4:1198750	−.480	.031	**SPON2**	Cell morphogenesis, cell motion, immune response, cell adhesion, axonogenesis
chr4:76533250	−.469	.044	CDKL2	Protein phosphorylation, signal transduction, reproductive developmental process
chr6:170496250	−.665	.030		
chr7:27141650	−.523	.030	**HOXA2**	Regulation of transcription, cell morphogenesis, cell motion, axonogenesis, neuron differentiation
chr7:100843550	−.684	.000	MOGAT3	Glycerol, alditol, and polyol metabolic process, lipid biosynthetic process
chr7:101454050	−.638	.030		
chr7:130522550	−.291	*.208*		
chr8:108555250	−.492	.030		
chr8:123888850	−.621	.029	**ZHX2**	Regulation of transcription, mRNA catabolic process, regulation of neuron differentiation
chr8:142389850	−.534	.030	lncRNA	
chr9:99251950	−.659	.030	HABP4	Regulation of transcription, platelet degranulation and activation, blood coagulation
chr9:140928850	−.557	*.091*	**CACNA1B**	Calcium ion transport, synaptic transmission, neurotransmitter secretion, locomotory behavior
chr10:88476150	−.492	.039	LDB3	Muscle alfa-actinin binding, zinc ion binding
chr11:1673750	−.477	.030	MOB2	Stimulates the autophosphorylation and kinase activity of STK38 and STK38L
chr11:69269150	−.479	.033		
chr13:49796550	−.580	.030		
chr13:10823895	−.496	.030	FAM155A	
chr14:10404775	−.506	.030	APOPT1; KLC1	Intrinsic apoptotic signaling pathway Microtubule motor activity and tubulin binding
chr16:88448350	−.480	.030		
chr17:64550	−.492	.031	RPH3AL	Exocytosis, response to drug, glucose homeostasis, regulation of G-protein signaling pathway
chr17:4755050	−.660	.019	**MINK1**	Protein phosphorylation, intracellular signaling cascade, synaptic transmission, cell–cell adhesion
chr17:43309250	−.601	.030	**FMNL1**	Substrate-dependent cell migration, regulation of cell shape, cortical actin cytoskeleton organization
chr17:75864350	−.504	.030		
chr18:61489650	−.568	.030		
chr19:55813850	−.483	.039	**BRSK1**	Intracellular signaling cascade, neurotransmitter secretion, neuron morphogenesis, axonogenesis
chr20:47087850	−.505	.030	TF binding site	
chr21:44715450	−.487	*.064*	TF binding site	
chr22:36874350	−.598	.030	**TXN2**	Electron transport chain, response to internal and external stimulus, response to axon injury

Table 2
Continued

ME marker, start position	Regression coefficients		Genome annotation	
	β	*p* value	Gene/locus	GO: biological process
chr22:50422050	−.546	.030	TF binding site	

Note. ME markers are presented in the order of their genomic localization. The epigenome-wide association study showed that all 38 ME markers were significantly associated with the Parental Acceptance–Rejection Questionnaire (PARQ) slope and the college version of the Behavior Assessment System for Children (BASC–CV) personal adjustment composite. A series of regression analyses was performed to test the modulating role of these markers in the relation between the PARQ slope and the BASC–CV personal adjustment composite. Regression coefficients and *p* values (adjusted for multiple comparisons using FDR method) for the 38 ME markers were estimated in 38 separate regressions of the BASC–CV personal adjustment composite on one ME marker and the PARQ slope, controlling for age, gender, and the number of substance-related and addictive disorders and psychiatric problems. $p > .05$ are italicized. Genes directly related to CNS development and functioning, as defined by Gene Ontology (GO) categories, are marked in bold. TF = transcription factor.

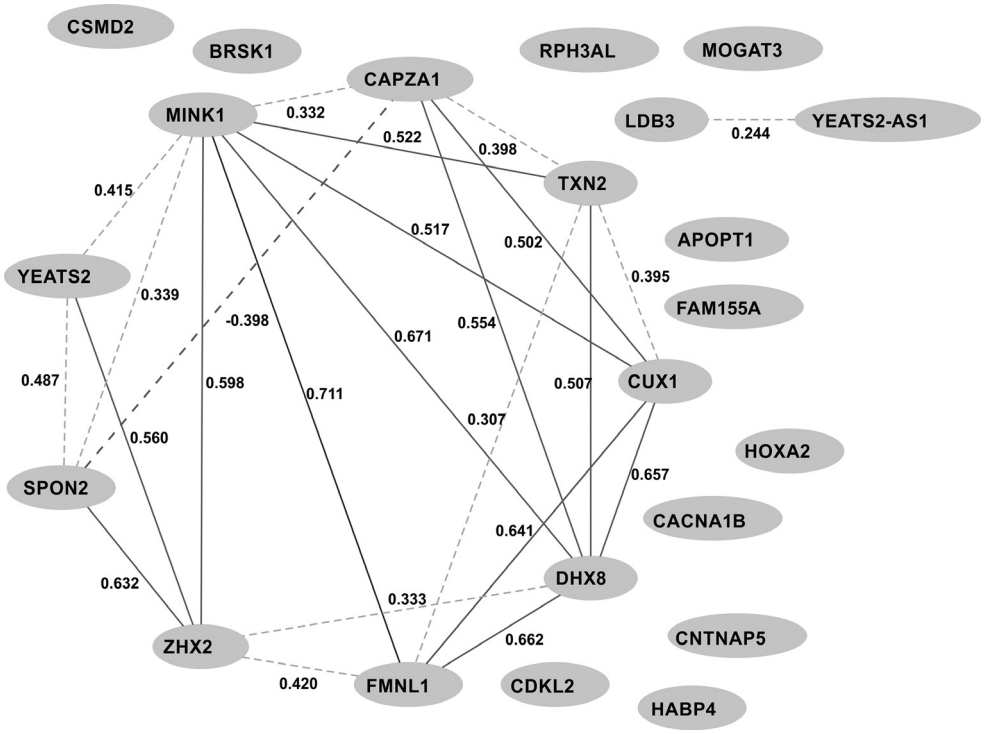

Figure 3. The tissue (blood)-specific coexpression network for the 23 genes associated with both the Parental Acceptance–Rejection Questionnaire slope and the college version of the Behavior Assessment System for Children Personal Adjustment Composite Personal Adjustment, constructed based on Genotype–Tissue Expression (GTEx) project database (Lonsdale et al., 2013) via a WebQTL resource (http://www.genenetwork.org). Interactions for absolute correlations above .50 are shown; Pearson pairwise correlations are represented. The nodes contain the symbols of genes. Notably, 12 of these 23 genes are known to be expressed in blood cells, and 9 of 12 active genes showed a high covariability in their expression levels among individuals.

Suderman et al., 2014; Weaver et al., 2004), there are few studies on the connection between the epigenome and family environment in the context of mother–child relationships, as well as studies on the connection between the epigenome and the change (or stability) of patterns in these relationships. Moreover, there is a gap in knowledge on the role of alterations and modifications in the epigenome driven by early experiences in relation to long-term behavioral outcomes. In our study, we attempted to provide a comprehensive picture of the potential modulating role of the epigenome,

namely, whole genome DNA methylation patterns, in established relations between perceived characteristics of parenting (in particular, stability and variation in parenting across time) and adult psychosocial outcomes.

Genome-wide DNA methylation patterns were assessed when participants were adults. The availability of data on perception of parenting and perceived maternal stress at three distinct developmental periods (middle childhood, adolescence, and young adulthood) allowed us to evaluate the connections between parenting, the epigenome, and adult behavior outcomes in a longitudinal study. Specifically, we evaluated the associations between epigenetic patterns and parenting indicators at different time points, averaged across all developmental stages, and with changes in the perception of parenting over time, from middle childhood to young adulthood.

The results of the EWAS showed that the trace left on the epigenome by subjective experiences (as reflected in perception) of parenting at each of three studied time points (from middle childhood to young adult) is better captured not through stable (i.e., absolute value) but dynamic (i.e., fluctuation between time points) characteristics of parenting. This finding may indicate that change in negative perception of parenting over time may exert an accumulating effect on the epigenome. We hope that other larger scale studies will provide additional evidence supporting this observation.

Specifically, significant associations were obtained between the PARQ slope and 818 ME markers located mostly in genes controlling GTPase activity, ATP binding, and metal ion binding, which are involved in a broad spectrum of biological processes and pathways. GTPases control differentiation during cell division, the synthesis of proteins and their translocation through membranes, and the activation of transmembrane receptors. ATP binding plays an important role in many metabolic and cellular processes, including mitochondrial energy metabolism, DNA synthesis, transcription activation, and cell signaling. In complicity, ion and ATP binding play a main role in the activation of signal receptors and, as a consequence, in signal transduction. Of particular interest is that all of these functional groups are related to intra- and intercellular signal transduction. Similar findings (i.e., alterations in the methylation of genes involved in the control of key cell signaling pathways) have been reported in association with adverse experiences early in life, such as child abuse (McGowan et al., 2009; Suderman et al., 2014), maternal deprivation (Meaney & Szyf, 2005; Naumova et al., 2012; Weaver et al., 2004), disadvantaged socioemotional position in childhood (Borghol et al., 2012), and others.

Besides the possible common effects on intra- and intercellular processes across different tissues, the enriched GO categories might be described in terms of cell-specific effects of the epigenetic alterations. Thus, the enrichment in GO categories related to the metabolism of purines (GTP and ATP) may indicate a disturbance in immune system functioning. There is evidence (Scheele, Marks, & Boss, 2007) that small GTPases (GO:0005083; Table 1) play an important role in maintaining normal immune system regulation of the functions of T cells, along with B lymphocytes and dendritic cells. Disturbances in purine nucleotide metabolism, such as a decrease in the activity of enzymes involved in these metabolic pathways and an increase in the A/G ratio in the lymphocytes may cause important malfunctions of the immune system to the point of developing serious disorders such as chronic lymphocytic leukemia (Carlucci et al., 1997). Thus, although preliminary, this finding contributes to the growing literature substantiating the connection between negative early environment and life-span health outcomes. Specifically, it suggests that the modulating role of the epigenome might be carried out, at least in part, through the alterations of the functioning of the immune system, which, in turn, trigger a cascade of negative organismic events resulting in poor developmental outcomes.

Due to a broad spectrum of biological processes controlled by genes that contain parenting-related methylation events, we can assume a direct or indirect involvement of these ME markers in shaping offspring's phenotype in the broadest sense, including behavioral phenotype. To investigate epigenetic factors as potential modulators of behavioral phenotypes examined through the prism of characteristics of adaptive and maladaptive behavior and psychological adjustment using the BASC–CV, we applied a set of multiple regression models to delineate associations between 818 parenting-associated ME markers and the BASC–CV composite and individual subscales. Of the 818 parenting-related ME markers, 38 showed significant negative association with the BASC–CV personal adjustment, and 33 of them passed conservative tests of a modulating role of their methylation states in the association between the temporal dynamics in perceived parenting from middle childhood to young adulthood and long-term behavioral outcomes, namely, per-

sonal adjustment in adulthood. Most of these 33 ME markers are related to 20 genes with known biological functions, including the genes directly involved in the control of brain development and functioning, and genes associated with various brain disorders, such as neuronitis (*HOXA2*, *MINK1*, *SPON2*, and *ZHX2*), neuropathy and schizophrenia (*CSMD2*, *LDB3*), and others.

It is important to note that we observed a number of suggestive (statistically borderline significant) associations between almost half of these 818 markers and such behavior outcomes as anxiety, hyperactivity, depression, and attention problems. These findings, first, are consistent with the literature that provides evidence associating psychiatric and neurodevelopmental disorders, such as depression and autism spectrum disorders, to alterations in DNA methylation (Dempster et al., 2014; Siniscalco, Cirillo, Bradstreet, & Antonucci, 2013). And, second, these observations indicate that the link between parenting-associated ME markers and behavior outcomes might be even more extensive, and that more associations might be detected in future studies.

Limitations and Future Directions

Several limitations in this study outline the need for further work. First, with regard to methodology, there is an insufficiency of current analytical techniques for conducting EWAS. The small sample size decreased the power to detect small effects and precluded the use of robust high breakdown point estimators or nonparametric approaches more suitable for accounting for the presence of participants with extreme values (i.e., perceptions of change in parenting). Although the method used for genome-wide profiling of the methylome (MBD-seq) has been proven to be highly informative and cost efficient, it captures only the genome regions with high densities of methylated CpGs. This introduces some constraints on detecting methylation levels of low-density regions or single methylation events that might be related to a phenotype. Moreover, although some way to correct for multiple comparisons was needed, perhaps the strategy used was overly conservative. It is possible that any positive findings that did not survive correction for multiple testing may resurface when different methods are applied (e.g., Bayesian adjustment through the assignment of prior probabilities to models).

Second, due to the low starting material, namely, the amount of T cells derived from a blood sample, we were not able to obtain RNA. A lack of data on the transcriptional activity of genes whose methylation statuses were found to be associated with negative parenting does not allow us to conclude, only to assume, that the found methylation marks may cause changes in gene activity. Therefore, the functional relation between DNA methylation and the expression levels of those genes need to be examined in further research involving a parallel analysis of methylation and transcription profiles in T cells.

Third, due to a lack of longitudinal epigenetic data we could not address important questions concerning the dynamics of epigenetic alterations associated with family environment—how to detect the most critical and sensitive developmental stages when changes occur, and how to explore their stability during development. Given well-established statements on the potentially reversible nature of DNA methylation changes, it is possible that by examining adult genomes we detected only especially stable changes that remain over the long term. At the same time we might have missed alterations that are short-lived but crucial at earlier stages of development. Also, longitudinal data on medical records and data on various facets of development will be needed if we are to determine the effects of DNA methylation alterations on developmental cascades and health outcomes during child development. All these questions hopefully should be addressed by future EWASs focused on longitudinal investigations of the epigenome at different stages of development in the context of early environments, taking into account potential confounding variables, such as data on prenatal, early postnatal, and other environmental factors.

Conclusions

Despite some limitations, our study provides evidence supporting an association between early social environment and the epigenome. Namely, the results of the study provide initial evidence of the differential associations between the offspring's epigenome and different facets of parenting. We found stronger associations with indicators of perceived parenting than with maternal reports of parenting stress. This suggests that changes in perceived parental rejection have a stronger association with the offspring's epigenome than measures of maternal parenting difficulties. However, since significant correlations were observed between both constructs that were self-reported by the participating offspring (i.e., personal adjustment and perceived maternal parenting), this finding highlights the possibility of shared method variance that

should be explored in future studies by including other-reported indicators of parenting and/or adult psychosocial outcomes in a multitrait, multimethod framework. The results of this study also imply that individual differences in the dynamics of offspring perceptions of parenting are crucial for understanding the factors of family environment that leave their mark on the epigenome.

In conclusion, this study presents a new longitudinal and multiphenotypic approach to investigating associations between family environment (e.g., parenting), epigenome (e.g., methylation), and long-term outcomes (e.g., psychosocial maladjustment). Together, the results contribute to a growing body of literature in the field of behavioral epigenetics on the association of parenting, methylome, and behavior, wherein the methylome may play a role as a connecting link or mediating mechanism between negative parenting and diverse facets of long-term psychosocial and behavioral adaptation.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's website:

Figure S1. Linear Regression Model That Relates the Mean Methylation Levels of 818 Parenting-Associated Markers to Age (A), Gender (B), Substance-Related and Addictive Disorders (SRAD; C), and Psychiatric Problems (D), Which Were Used as Independent Covariates in the Multiple Linear Regression Models in the EWAS (see Methods)

Table S1. Demographic Information and Characteristics of Substance Abuse (SRAD) and Psychiatric Problems of the Participants of the Study

Table S2. Descriptive Statistics for Perceived Parental Rejection (PARQ) and Maternal Stress (PSI-SF)

Table S3. Descriptive Statistics for Psychosocial Adjustment (BASC-CV) for the Participants of the Study at the Third Time Point

Table S4. MBD Pull Down Experimental Conditions and ME-DNA QC; MBD-Seq Outcomes and the Results of Alignment to the Reference Human Genome hg19

Table S5. DNA Methylation Markers Significantly Associated With the Dynamic Score of Perceived Parental Rejection (PARQ Slope) and the BASC-CV Personal Adjustment Composite Score

Table S6. The List of 565 Genes Which Have Shown Significant Associations With the Dynamic Score of Perceived Parental Rejection (PARQ Slope)

Table S7. DAVID GO Annotation of 565 Genes Associated With the PARQ Slope

Appendix S1. Methods