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Full Length Article

Epigenetic differences in inflammation genes of monozygotic twins are related to parent-child emotional availability and health

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ABSTRACT

The inflammatory response is an immune defense engaged immediately after injury or infection. Chronic inflammation can be deleterious for various health outcomes and is characterized by high levels of pro-inflammatory markers such as C-reactive protein (CRP), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α). A large body of research demonstrates these inflammatory markers are responsive to stress and quality of social relationships throughout the lifespan. For example, the quality of the early parental bond predicts various health outcomes and may be driven by changes in immune function. Epigenetic processes, such as DNA methylation, may be one mechanism by which early social experiences shape immune functioning. The present study used a monozygotic twin difference design to assess if mother-reported emotional availability at 1 year and 2.5 years predicted immune gene methylation at 8 years of age. Further, we assessed if inflammation gene methylation was related to general health problems (e.g. infections, allergies, etc.). We found that mother-reported emotional availability at 1 year, but not 2.5 years, was related to methylation of various immune genes in monozygotic twins. Furthermore, twin pairs discordant in health problems have more difference in immune gene methylation compared to twin pairs concordant for health problems, suggesting that methylation of immune genes may have functional consequences for general health. These results suggest that the emotional component of attachment quality during infancy contributes to immune epigenetic profiles in childhood, which may influence general health.

1. Introduction

The inflammatory response is an immune defense engaged immediately after injury or infection. While inflammation is a necessary process, chronic inflammation can be deleterious to health. Chronic inflammation is characterized by high levels of pro-inflammatory markers such as C-reactive protein (CRP), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) (Lund et al., 2011). Markers of chronic inflammation are associated with various deleterious health conditions throughout development. For example, obesity indices such as body mass index (BMI) and adiposity are consistently correlated with increases in circulating levels of IL-6 and CRP (Himmerich et al., 2006; Panagiotakos et al., 2005; Rexrode et al., 2003). Higher CRP is also associated with increased sensitivity to viral infections in children, and lower levels of self-reported general health in adulthood (Dowd et al., 2010; Carpenter et al., 2012). Elevated levels of IL-6 and CRP also predict the development of type 2 diabetes, and are related to several cardiovascular risk factors and certain cancers (Pradhan et al., 2001; Bermudez et al., 2002; Park et al., 2005; Kumar et al., 2016). Later in life, higher circulating levels of IL-6, TNF-α, and CRP in CSF and plasma are associated with Alzheimer's and Parkinson's disease (Tan et al., 2007; Blum-Degena et al., 1995). Importantly, much research demonstrates these inflammatory markers are responsive to early life trauma (Baumeister et al., 2016).

A large body of literature suggests that social experiences, such as stress and parental attachment, during childhood program immune system functioning throughout life. For example, retrospective reports of childhood trauma and maltreatment are related to immune functioning in adulthood, such as high sensitivity CRP and higher IL-6 and TNF-α (Baumeister et al., 2016; Danese et al., 2007; Fagundes et al., 2013; Miller and Chen, 2010; Kiecolt-Glaser et al., 2011; Smith et al., 2011; Slopen et al., 2010) A recent meta-analysis including a sample of 16, 870 individuals for CRP, 3751 individuals for IL-6, and 881 individuals for

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TNF-α showed that individuals exposed to childhood trauma had significantly elevated baseline peripheral levels of all three inflammation markers (Baumeister et al., 2016). Since increased inflammation is a risk factor for various physical and mental disorders, childhood trauma can be conceptualized as a subtle effect that is likely to have a significant impact on physical and mental health.

In addition to trauma, an important aspect of childhood social experience is parent-child attachment. In the first year of life, children form primary attachments to their primary caregivers who provide them protection and care essential to their survival (Ainsworth, 1989). A child classified as securely attached uses his/her parent as a safe haven during times of distress and as a secure base from which to explore the environment when not distressed (Kerns and Brumariu, 2014). Attachment quality in childhood is highly predictive of interpersonal relationship quality in adulthood, and both are consistently associated with physical health and mortality (Hazan and Shaver, 1987; Cohen, 2004; Holt-Lunstad et al., 2010). Further, a groundbreaking study following participants from infancy to age 32 found that insecurely attached infants were more likely to experience an inflammation-related illness (e.g., heart disease, diabetes, stroke) as adults, even after controlling for other known risk factors (Puig et al., 2013). Conversely, children with secure attachments report fewer symptoms of asthma and produce a lower cytokine response after an immune challenge (Ehrlich et al., 2018). High maternal warmth, a characteristic often associated with secure attachment, also predicts lower levels of IL-6, and buffers the negative effects of early life stress on various stress, cardiac, and metabolic pathways (Chen et al., 2011; Carroll et al., 2013; Farrell et al., 2017, 2019). Taken together, this body of research demonstrates that parent-child relationships can shape immune functioning throughout development. Still, understanding the mechanistic properties that "program" long-term immune function remains an important endeavor.

Epigenetic processes, such as DNA methylation, may be one mechanism by which early social experiences shape the molecular biology of inflammatory regulation and by extension immune system function and health. Early life stress is related to dysregulation of the slow-acting neuroendocrine stress response of glucocorticoid secretion, the hypothalamic pituitary adrenal (HPA) axis. Accumulating human and animal research suggest that childhood trauma alters DNA methylation of HPA genes which leads to dysregulated levels of glucocorticoids (Dadds et al., 2015; Carpenter et al., 2011; Essex et al., 2011; Heim and Nemeroff, 2001; van der Knaap et al., 2014; Weaver et al., 2004). HPA and immune function are highly interrelated as glucocorticoids assert both permissive and stimulatory effects on the immune system, depending on the specific conditions (Bellavance and Rivest, 2014). Glucocorticoids initially have immunosuppressive and anti-inflammatory functions, however, chronic glucocorticoid exposure leads to immune dysregulation, suppressing some immune responses while enhancing others (Chrousos, 2002; Padgett and Glaser, 2003; Robles et al., 2005). Recent research has also linked early life stress to methylation of immune genes. Prenatal maternal stressors like famine, natural disasters, and maternal anxiety are associated with lower methylation of numerous immune function genes (Heijmans et al., 2008; Vangeel et al., 2015; Cao-Lei et al., 2014). Early life stressors including foster care and child maltreatment are associated with differential methylation of genes in immunity pathways in adulthood (Bick et al., 2012; Yang et al., 2013; Naumova et al., 2012). In contrast, positive social experiences may also influence immune and HPA gene methylation, for example, child report of maternal warmth positively predicted *NR3C1* expression in peripheral cells in children aged 10–17 years old (Stanton et al., 2017). Others found a supportive family environment during adolescence may buffer against the effects of racial discrimination on epigenetic aging in young adulthood (Brody et al., 2016). Together, these results make a compelling case that the quality of parental attachment early in life may influence immune gene methylation and overall health throughout life.

Based on this prior research, we hypothesized that the emotional component of the attachment relationship, emotional availability, influences inflammation gene methylation and general health. Emotional availability can be defined as an individual's emotional responsiveness and attunement to another's needs and goals (Biringen and Easterbrooks, 2012). Importantly, emotional availability is an attachment relationship measure more than a parent or child personality measure (Bretherton, 2000). Because DNA methylation is both genetically driven and reactive to the environment, genetic confounds in epigenetic research are important to consider. Causal inferences about parental environment on epigenetic measures cannot be established without controlling for genetic influences, usually with a family- or twin-based design (Sherlock and Zietsch, 2016). For this reason, we used a monozygotic (MZ) twin difference design to control for genetic effects on DNA methylation. Because MZ twins largely share the same DNA, any differences in DNA methylation are most likely attributable to environmental influences. We tested the association between primary caregiver-reported emotional availability (EA) at two key points in early development, age 1 and 2.5 years, with DNA methylation of the inflammation genes *IL6*, *CRP,* and *TNF* at eight years old. Further, we hypothesized that twin pairs discordant in general health would be more different in immune gene methylation compared to twins concordant in general health at eight years old.

2. Methods

2.1. Sample

Participants were recruited from state birth records for a longitudinal twin study investigating genetic and environmental influences on childhood health (Lemery-Chalfant et al., 2013, 2019). We recruited a sub-sample of monozygotic (MZ) twins ($N = 96$; 51% male; 50% Non-Hispanic White, 14.6% Hispanic/Latinx, 8.3% African American, 4.2% Asian American), $M_{age} = 8.5$ years, $SD = 0.45$. Seventy-one percent of primary caregivers reported currently being married and all were mothers. Total household income ranged from $6400 - $300,000 USD ($M = $97,057, $SD = $64,893) and 21% of families met Federal Medicaid Eligibility based on 2016 standards (Arizona Median Household Income = $53,510, US Census Bureau). All study procedures were approved by institutional review boards and are in accordance with the Helsinki Declaration of 1975.

2.2. Mother-reported emotional availability

Primary caregivers completed a 28-item, abridged version of the EA Self-Report when the twins were both 1 year ($M = 12.5$ months, $SD = 1.06$) and 2.5 years ($M = 2.55$ years, $SD = 0.08$) of age over the phone (Vliegen et al., 2009; Biringen, 2004). Items were rated on a five-point Likert scale ranging from "almost never" to "almost always" and were asked separately for each twin. A higher score indicated higher mother-reported EA in the parent-child relationship. In our study, Cronbach's alpha was 0.721 and 0.806 at 1 and 2.5 years, respectively. The use of this self-report measure occurs with prior permission from the developer.

2.3. General health composite

A general health composite was computed using items from the parent-reported MacArthur Health and Behavior Questionnaire (HBQ) (Essex et al., 2002). Items endorsing asthma, chronic/recurrent lung disease, repeated, persistent ear, urinary, respiratory infections, and bad allergies requiring doctor visits and frequent medication were summed into one composite of health problems. In our sample, the composite had a range of 0–4, with a maximum possible range of 0–6. Twin pairs with a difference score of "0" were categorized as "Concordant in General Health" and twin pairs with a difference score >0 were categorized as "Discordant in General Health".

2.4. Methylation

Buccal cells were collected with Mawi iSWAB DNA collection tubes (Mawi DNA Technologies LLC, Hayward, CA) during an eight-year home visit. DNA was extracted with a standard isolation kit (Qiagen, Hilden, Germany). Sample yield and purity were assessed spectrophotometrically using a NanoDrop ND-1000 (ThermoScientific, Wilmington, DE) and using Qubit fluorometric methods. Approximately 500 ng of DNA was treated with sodium bisulfite using the EZ-96 DNA Methylation Kit (Zymo Research, Irvine, CA). DNA methylation was quantified using the Infinium MethylationEPIC BeadChip run on an Illumina iScanSystem (Illumina, San Diego, CA). Raw IDAT files were exported for pre-processing in R with the minfi package (Aryee et al., 2014). We applied a filter to remove probes located on the sex chromosomes. Data was subjected to quality control analyses, which included quantile normalization, checking for sex mismatches, and excluding low-intensity samples ($p < 0.01$). Three samples did not pass our quality control pipeline. Data were normalized and annotated with Illumina CpG site probe names. Using the R package EpiDISH (Epigenetic Dissection of Intra-Sample Heterogeneity, 3.8) RPC method, we included the proportion of estimated epithelial cells as a covariate in our statistical models (m = 69%) (Teschendorff et al., 2017; Houseman et al., 2012). Array number was included as a covariate in all analyses to control for batch effects.

We interrogated candidate genes by identifying probes on the array that were annotated to pre-specified genes. The immunity genes we selected were identified by prior associations between methylation or expression and stress. We verified that candidate genes are expressed in esophagus mucosa, suggesting they may *have functional consequences* sue (https://www.gtexportal.org/). Average beta values were calculated by dividing the methylated probe signal intensity by the sum of methylated and unmethylated probe signal intensities. Average beta values range from 0 (completely unmethylated) to 1 (fully methylated) and provide a quantitative readout of relative DNA methylation for each CpG site. The M-value was calculated as the log2 ratio of the intensities of methylated probe versus unmethylated probe. An M-value close to 0 indicates a similar intensity between the methylated and unmethylated probes, which means the CpG site is about half-methylated. Positive M-values indicate that more molecules are methylated than unmethylated, while negative M-values mean the opposite (Du et al., 2010).

2.5. Statistical analysis

We used principal components analysis (PCA) to reduce data dimensionality on our candidate genes, which had 18-52 associated CpG probes. This procedure allowed us to use gene methylation summary statistics to characterize the data and reduce the number of statistical tests to avoid Type 1 error. PCA is an established technique that has been used to reduce high-dimensionality of methylation data (Dadds et al., 2015; Lewis et al., 2019; Liu et al., 2010; Model et al., 2001). PCA projects data into new orthogonal directions corresponding to the directions of maximum variance. PCA appropriateness was assessed with the Kaiser-Meyer-Olkin Measure of Sampling Adequacy and the Barlett's Test of Sphericity. All PCA models had CpG sites with positive loadings (representing higher methylation) and sites with negative loadings (representing lower methylation). We extracted the first component after removing all sites with loading values between −0.3 and 0.3. Component scores were computed as the raw CpG M-values weighted by the factor loadings. M-values were used for methylation analysis as has been recommended (e.g. reduces homoscedasticity).

MZ twin difference scores were computed from methylation components, EA Self-Report composites, and general health composites. We used linear multiple regression analysis controlling for sex, methylation array, and cell count. Differences in EA Self-Report were used as predictor variables and differences in methylation and were used as dependent variables. We report the standardized beta value for all regression results with the p and corresponding FDR corrected p values.

Table 1
Intra-twin correlations.

EA	*r*	*p*
1 year	0.950	<0.001
2.5 years	0.926	<0.001
Gene Methylation	***r***	***p***
IL6	0.407	0.006
CRP	0.299	0.006
TNF	0.500	0.001

We used independent samples t-test to compare methylation differences between twin pairs discordant in general health (n = 7 pairs) and concordant in general health (n = 29 pairs). All analyses were conducted in SPSS 25. For all variables, > 3 standard deviations from the mean were considered outliers and dropped from the analysis. For all variables, 0–4 outliers were removed.

3. Results

3.1. EA self-report

Intra-twin EA Self-Report composites were correlated at 1 year ($r = 0.95, p < 0.001$) with 47% of twins with a difference score > zero. At 2.5 years, intra-twin EA Self-Report composites were correlated ($r = 0.93$, $p < 0.001$) with 70% of twins with a difference score > zero (Table 1). EA Self-Report at 12 and 30 months are significantly correlated ($r = 0.49$, $p = < 0.001$).

3.2. Principal components analyses

All PCA eigenvalues and variance explained are reported in Table 2. For all analyses, Kaiser-Meyer-Olkin Measure of Sampling Adequacy ≥ .80, indicating data was suited for factor analysis. For all analyses, Barlett's Test of Sphericity was significant at $p \leq 0.001$, indicating that the observed correlation matrix was not likely observed by chance. The loadings and site identifiers for all genes of interest are reported in Supplementary Tables. Positive loadings indicate sites that have more methylation with EA Self-Report whereas negative loadings indicate sites that have less methylation with EA Self Report.

3.3. Associations between EA self-report and immune gene methylation

Using FDR correction and multiple linear regression controlling for sex, array, and cell count we found MZ differences in EA Self-Report at 1 year predicted differences in the methylation of *IL6* ($\beta = 1.423$, $p = 0.046$), and *TNF* ($\beta = 1.736$, $p = 0.046$), but not *CRP* ($\beta = 2.145$, $p = 0.121$; Table 3). Differences in EA Self-Report at 2.5 years did not predict differences in methylation of any immune gene tested (all *ps* > 0.05; Table 3).

3.4. General health, EA self-report, and immune gene methylation

Twin pairs discordant in general health had a significantly larger difference in EA Self-Report at 1 year compared to twin pairs concordant in general health ($t\ (28) = -2.547$, $p = 0.017$; Table 4). Difference in EA Self-Report at 2.5 years was not significantly different between groups.

After FDR correction, twin pairs discordant in general health had larger differences in the methylation of *IL6* ($t\ (30) = -2.640$, $p = 0.027$) and *CRP* ($t\ (31) = -2.494$, $p = 0.027$ but not TNF; Fig. 1). Cohen's d effect sizes for all t-tests are reported (Table 4).

4. Discussion

This study found that mother-reported EA at 1 year, but not 2.5 years, is related to methylation of *IL6* and *TNF* but not *CRP* in middle childhood

Table 2
Immune gene methylation PCA results.

Gene	CpG Probes	Component Probes	Eigenvalue	% Variance
IL6	52	28	9.537	34.06
CRP	18	9	2.534	28.16
TNF	27	22	11.193	50.88

using a MZ twin difference design. Furthermore, twin pairs discordant in health problems have more difference in *IL6* and *CRP* gene methylation compared to twin pairs concordant for health problems, suggesting that methylation of inflammation genes may have functional consequences for health. These findings are especially important because there is a substantial body of literature describing the association between inflammatory biomarkers and negative emotional factors in childhood (e.g., abuse and neglect) (Baumeister et al., 2016). However, less is known about the relationship between attachment and immune function. Further, the burgeoning field of epigenetics has largely focused on detrimental life events and disease vulnerability without much attention to measures that include positive social experiences promoting health. Our results add to this literature suggesting that attachment to the primary caregiver may shape health through epigenetic regulation of immune activity.

These findings converge with prior studies linking early social experiences with health outcomes across the lifespan. It has long been known that children raised in high conflict families that are low in emotional warmth and parental involvement are at risk for emotional and behavioral problems throughout life (Sander and McCarty, 2005). More recently, mounting evidence suggests that these early social experiences also play a role in physical health issues such as metabolic disorders. For example, the well-known Adverse Childhood Experiences (ACE) Study with more than 17,000 adults found that rates of cardiovascular disease, autoimmune disorders, and premature death were 1.5–2.0 times higher among respondents who were exposed to familial violence, abuse, and

Table 3
Standardized beta estimates for regression analyses. The bolded text highlights significant results after FDR correction.

Gene	Emotional Availability at 1 year				Emotional Availability at 2.5 years			
	β	SE	*P*	*FDR p*	*β*	SE	*p*	*FDR p*
IL6	**1.423#**	**0.588**	**0.022**	**0.046**	1.019#	0.546	0.075	0.224
CRP	2.145#	1.342	0.121	0.121	0.740#	1.286	0.571	0.571
TNF	**1.736**	**0.762**	**0.031**	**0.046**	0.718	0.740	0.342	0.512

FDR: false discovery rate; # = Cell Count < 0.05.

Table 4
Twins Discordant in Health are More Different in Immune Gene Methylation. The bolded text highlights significant results after FDR correction.

	Twin-Pairs Discordant in General Health		Twin-Pairs Concordant in General Health		*t-test*			Effect Size
Difference Scores	*M*	*SD*	*M*	*SD*	*t*	*p*	FDR p	Cohen's d
EA 1 year	0.134	0.121	0.051	0.055	**−2.547**	**0.017**	–	0.88
EA 2.5 years	0.101	0.137	0.108	0.134	0.111	0.912	–	0.05
IL6	1.211	0.925	0.550	0.442	**−2.640**	**0.013**	**0.027**	0.911
CRP	0.999	0.614	0.505	0.422	**−2.494**	**0.018**	**0.027**	0.937
TNF	0.833	0.642	0.554	0.326	−1.593	0.122	0.122	0.548

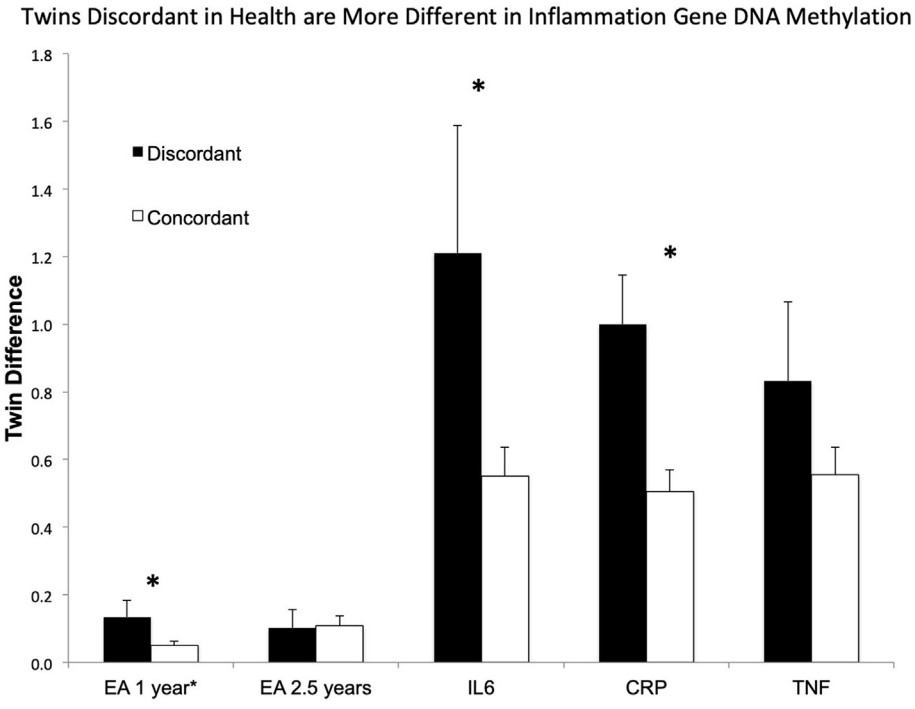

Fig. 1. Intra-pair differences of mother-reported emotional availability and inflammation gene DNA methylation in monozygotic (MZ) twins. We plot comparisons between twins concordant and discordant in general health. This data demonstrates twins discordant in health are more different in mother-reported emotional availability (EA) from 1 year old but not 2.5 years old. The data also shows twins discordant in health are more different in methylation of IL-6 and CRP but not TNF compared to twins concordant in health (* FDR corrected $p < 0.05$). Error bars represent standard error of the mean (SEM).

neglect as children than among those who were not (Anda et al., 2006; Dube et al., 2009; Dong et al., 2004). More specifically, much research suggests that insecure parental attachment in infancy and early childhood has the potential to impair physical health throughout the lifespan (Maunder and Hunter, 2008). Several studies suggest that the pathway from unfavorable circumstances in childhood to increased disease risk is through increased inflammatory activity (Danese et al., 2007; Miller and Chen, 2007). Our findings extend this prior research by revealing a mechanistic insight such that the mother-reported emotional availability component of attachment may shape immune function and subsequent health through epigenetic processes. Importantly, an emerging body of evidence links epigenetic mechanisms in asthma, inflammation, and lung-disease, further offering support for the functional consequences of epigenetic modifications arising from parental care (Ji et al., 2016; Raghuraman et al., 2016; Willis-Owen and Moffatt, 2012). Because we used the MZ twin difference design, which controls for genetic confounds, we can say with more confidence that the relationship we found between the attachment relationship and child health is environmentally driven.

Much evidence supports the basic tenets of the prevailing hypothesis that harsh early-life family climates engender a dysregulated glucocorticoid response to stress and proinflammatory phenotypes via desensitization or down regulation of the glucocorticoid receptor (Miller and Chen, 2007, 2010; Cohen et al., 2012). Our results add to this model the possibility that early attachment relationships contribute to later immune function through epigenetic modifications of inflammation genes as well. However, it is currently unknown how methylation levels respond to early psychosocial cues. Because the quality of the attachment relationship influences diurnal cortisol and stress reactivity, the cortisol bound glucocorticoid receptor may shape DNA methylation through action as a transcription factor for thousands of genes, including DNA methyltransferase (DNMT) (Yang et al., 2013; Thomassin et al., 2001; Pape et al., 2018; Wiench et al., 2011; Le et al., 2005). Accordingly, childhood social cues, such as the quality of the attachment relationship, may shape methylation patterns of inflammation genes through influencing cortisol patterns and subsequent cortisol bound GR activity.

It is unclear why we found mother-reported EA at one year but not 2.5 years was associated with immune methylation and childhood health. Perhaps this pattern of results is due to a sensitive period of immune epigenetic malleability during infancy. Work in developmental neuroscience indicates that prenatal and postnatal brain development has heightened sensitivity to environmental influences (Stiles and Jernigan, 2010). During these periods of increased plasticity, the environment plays a major role in shaping long-term brain structure and function, and subsequent behavior (Als et al., 2004). More recently, neuroepigenetic studies in rodent and human studies demonstrate that one mechanism of long-term environmental effects is through altering the epigenome and downstream expression of proteins underlying brain structure and function, especially with the HPA system (Roth and Sweatt, 2011). The current results suggest that immune genes may share a similar epigenetic sensitive period as HPA genes. Because the immune system gradually matures and "acquires memory" by exposures during infancy (Simon et al., 2015), immune epigenetic programming early in life seems plausible. This would align well with the established knowledge that early life stress influences long-term HPA and immune function. The current results suggest the immune epigenetic sensitive period may be short lived and that the epigenetic profile may in turn confer either risk or resilience to later immune issues such as asthma and infections. However, these effects will need to be replicated in a larger study.

Researchers have long been interested in how childhood psychosocial factors affect long-term immune function (Moore, 1989). More recently, studies have assessed epigenetic mechanistic pathways between negative childhood experiences and immune function. For example, prenatal stressors like famine, natural disasters, and maternal anxiety along with early life stressors including foster care and child maltreatment are associated with differential methylation of genes in immunity pathways (Heijmans et al., 2008; Vangeel et al., 2015; Cao-Lei et al., 2014; Bick et al., 2012; Yang et al., 2013). It is then interesting to extend this literature to assessing the quality of the attachment relationship on the immune epigenome. Recent literature reflects a growing interest on the potential impact of relationship quality and secure attachment on health, however this work has primarily focused on adult relationships (Gouin et al., 2009; Jaremka et al., 2013; Ironson et al., 2018; Seligman, 2008), with one exception (Puig et al., 2013). We contribute findings suggesting that mother-reported emotional availability may shape immune epigenetics and general health in childhood. This is of particular interest in the context of disorders with a later onset in life, such as heart disease and Alzheimer's disease, which have been related to chronic inflammation (Baker et al., 2011; Akiyama et al., 2000). Thus, our findings warrant an increased focus on secure attachment and beneficial early social relationships in the context of epigenetics and health across the lifespan.

While we are one of the first to assess the influence of infant-parent relationship quality on immune gene methylation and general health, there are limitations to consider. For example, despite our robust findings, our sample size is relatively small. However, in the context of MZ twin difference designs our sample size is well within the typical range (Gervin et al., 2012; Ribel-Madsen et al., 2012; Fraga et al., 2005; Kaminsky et al., 2009), but replications in larger nationally representative samples are necessary to confirm our conclusions. We also could not verify the parent-reported general health composite with medical records, although these types of health checklists have been shown to be valid in previous studies (Essex et al., 2002). Because we were unable to obtain T cells, we assessed DNA methylation of inflammation genes in buccal cells. While future studies need to replicate our findings in isolated T cell populations, work before us has demonstrated prenatal stress had similar effects on DNA methylation of immune genes across T cells, peripheral blood mononuclear cells, and saliva cells (Cao-Lei et al., 2014). Even with these limitations, we observed moderate effect sizes despite the seven-year gap between attachment assessments and physical health and methylation measures (Cao-Lei et al., 2014). Thus, using buccal swabs for DNA methylation studies holds great promise for large-scale studies in which obtaining T cells is rarely feasible, particularly when collecting from young children.

In summary, quality of attachment relationships may influence mental and physical health throughout the lifespan. We present results suggesting that DNA methylation of inflammation genes may be responsive to infant attachment. Further, our results suggest inflammation epigenetics have functional consequences for health. Lastly, because we used a twin difference design, we can be more confident that these relationships are environmentally driven and not genetically confounded. Given substantial evidence that childhood psychosocial factors impact immune function and risk for detrimental health outcomes, understanding how experiences "get under the skin" remains an important inquiry for health. Our results add to a growing literature supporting an association between early social experiences, immune gene epigenetics, and general health.

Declaration of competing interest

The authors have no potential or actual conflicts of interest to disclose.

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Appendix A. Supplementary data

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