Examining the potential of epigenetic age to mediate the relationship between adverse childhood experiences and locus of control using the ALSPAC cohort

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Examining the Potential of Epigenetic Age to Mediate the Relationship Between Adverse Childhood Experiences and Locus of Control Using the ALSPAC Cohort

by

Christopher Reddy

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ABSTRACT

Adverse childhood experiences (ACEs) are negative events and environments (e.g., poverty) that occur during childhood and are known to undermine health and wellbeing. Adverse childhood experiences are related to cognitive outcomes in populations of children. A specific area of cognition associated with ACEs is locus of control (LoC). While the association between ACEs and LoC is clear, what is not well understood is how—that is, through what mechanism—ACEs influence LoC. The purpose of this research was to evaluate epigenetic age as a potential mechanism connecting ACEs with LoC. To address this hypothesis, a mediation model was evaluated using the Accessible Resource for Integrated Epigenomic Studies (ARIES) cohort. The final analytic sample included $N = 894$ children. In this study, epigenetic age did not mediate the relationship between poverty and locus of control, indirect effect $\beta = -0.002$, 95% bootstrap CI [-0.012, 0.007]. The associations remained null when genders were evaluated independently and when the components of the poverty variable—financial difficulties and neighborhood stress—were evaluated independently. The null findings suggest that the severity of adversity in childhood matters, poverty may take time to influence epigenetic age and LoC, some poverty indicators are more influential than others, epigenetic age is an idiosyncratic variable, females may be more sensitive to adversity, and other biological mechanisms linking poverty and locus of control are worth considering.
DEDICATION

To Linda and Ed Reddy, who kept my sisters and I adversity-free as children.

To Jennifer Reddy, who is keeping my son’s childhood adversity free.

To all the children experiencing adversity, hang in there.
ACKNOWLEDGEMENTS

Great instructors teach by doing. In that respect, Dr. Schlomer is one of the best. Thank you for your patience, words of encouragement, mentorship, and avoidance of sugar-coating.

Dr. Appleton and Dr. Yan, thank you for making the thesis experience more about learning than about managing stress. In other words, thank you for modeling how research is conducted and guiding me through the many mistakes. Thank you, Dr. Appleton, for teaching me how to connect childhood adversity with outcomes that matter. Thank you, Dr. Yan, for teaching me how to be a professional.

Thank you, Dr. Colvin and Dr. Moeyart, for making statistics not only easier, but interesting. The language of science is truly math, and the language of math spoken by Dr. Colvin and Dr. Moeyart is easy to understand. Thank you, Dr. Andrade, for teaching me how to self-regulate and communicate with clarity.

This Ph.D belongs to my wife, who has essentially raised our son by herself, while I pursued this selfish endeavor. Support is insufficient a word to describe her influence. Scaffold—or perhaps cornerstone—starts nudging in the right direction.

Last, I am extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

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(http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf). The DNA methylation data from the ARIES project used in this study were supported by the following grants: BBSRC (BBI025751/1 and BB/I025263/1), IEU (MC_UU_12013/1 & MC_UU_12013/2 & MC_UU_12013/8), NICHD (R01HD068437), NIH (5RO1AI121226-02, and CONTAMED EU collaboration Project (212502).
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CHAPTER ONE

INTRODUCTION

Statement of the Problem

Adverse childhood experiences (ACEs) are negative events and environments (e.g., poverty) that occur during childhood and are known to undermine health and wellbeing. Physical and sexual abuse, emotional abuse, parental substance abuse, exposure to violence (Hughes et al., 2017; Liming & Grube, 2018) and indicators of poverty (Hughes & Tucker, 2018) are commonly assessed ACEs. Adverse childhood experiences are prevalent and are important to study because they are related to detrimental outcomes. How—that is, through what mechanism—ACEs influence detrimental outcomes is not well understood and is the topic of this research.

ACEs: Prevalence and Relationship With Detrimental Outcomes

Adverse childhood experiences are common and occur often. The Adverse Childhood Experiences (ACE) Study (Felitti et al., 1998) not only coined the phrase “adverse childhood experience,” but also brought to light the prevalence of ACEs by reporting that in a large sample—8,056 adults—about half had experienced at least one ACE by the age of 18. As another exemplar demonstrating the prevalence, Edwards et al. (2003) found that of an adult population of 8,667, 43% retrospectively reported having experienced at least one ACE. Lastly, consider a meta-analysis conducted by Hughes et al. (2017) as a more recent example: 57% of a pooled sample of adults (N = 253,719) retrospectively reported at least one ACE. Indeed, in some at risk samples, the percentage of people reporting at least one ACE approaches 100% (see Clarkson Freeman, 2014 or Flaherty et al., 2013). In light of these reports, ACEs may be the norm rather than the exception during childhood. Their diffuse pervasiveness is worrisome not
only because children are being harmed, but also because ACEs may undermine a person’s health, wellbeing, and cognition.

Adverse childhood experiences are strongly related to detrimental behavioral, physical, and cognitive outcomes in both adolescent and adult populations. For example, risky sexual behavior in adulthood (Hughes et al., 2017) and number of teenage pregnancies (Hillis et al., 2004) are associated with ACEs. Or consider that illnesses requiring a doctor (Schilling et al., 2007) and cancer diagnosis (Holman et al., 2016) are related to ACEs. Lastly, ACEs were also found to be associated with an anxiety and depression mental health score (Edwards et al., 2003), depression in teenagers (Schilling et al., 2007), and locus of control (Bosma et al., 1999; Culpin et al., 2015).

**Adverse Childhood Experiences and Locus of Control**

A specific area of cognition associated with ACEs is locus of control (LoC). Locus of control refers to how a person thinks about the consequences (i.e., rewards, punishments) of their own actions (Rotter, 1966). A person with an external LoC attributes the consequences of their actions to external factors outside of their control, such as luck or fate. A person with an internal LoC attributes the consequences of their actions to factors within their control, like their own behavior or characteristics (Rotter, 1966). An external LoC is associated with a myriad of negative consequences (Twenge et al., 2004). There are several factors that shape a person’s LoC, one of which is ACEs.

Adverse childhood experiences are associated with an external LoC (Carton et al., 2021; Hovens et al., 2016; Pedron et al., 2021); a person who experiences ACEs is more likely to attribute the cause of life events to luck or fate. That ACEs are associated with an external LoC is worrisome for three reasons. First, LoC can influence how the person thinks about other
detrimental outcomes of ACEs. This could subsequently impact interventions whose aim it is to ameliorate the detrimental state (e.g., Anastasiou et al., 2015). For example, it was reported that individuals with both hypertension and an internal LoC were more likely to adhere to a treatment regime than patients with an external LoC and hypertension (Omeje & Nebo, 2011). The inference to draw from this study is that people with an internal LoC consider their state of health as something they can influence and thus take steps to ameliorate their illness.

Second, LoC itself is not only an outcome influenced by ACEs, but also can act as a mechanism connecting ACEs with other detrimental outcomes (Fisher et al., 2013; Hovens et al., 2016). For instance, within the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, the cohort utilized for this study, it was reported that 34% of the association between childhood SES and depression in young adulthood was explained by an external LoC measured in adolescence (Culpin et al., 2015).

Third, LoC is related to educational outcomes, a particularly important feature of childhood and development (Findley & Cooper, 1983). For example, LoC has strong associations with academic self-concept, that is, the metacognitive practice of thinking about one’s own academic ability (Albert & Dahling, 2016).

To summarize thus far: Many children experience ACEs. Adverse childhood experiences are associated with many physical, behavioral, and cognitive outcomes that undermine health, wellbeing, and cognition. One such cognitive outcome is LoC. The relationship between ACEs and LoC is worth investigating because LoC is a facet of cognition that acts as a “hub” which directs an incalculable number of actions and behaviors. That LoC is also related to other detrimental outcomes, mediates associations between ACEs and other detrimental outcomes, and
plays a significant role in academic success further emphasizes the rationale for a deeper investigation into the relationship between ACEs and LoC.

While the association between ACEs and LoC is clear, what is not well understood, however, is how—that is, through what mechanism—are ACEs influencing LoC. Or, phrased differently, how do ACEs “get under the skin” to influence a facet of cognition like LoC?

**How Do ACEs Get Under The Skin?**

One proposed way that ACEs can get under the skin is through modifications to physiology. In other words, ACEs cause physiological adjustments (Hertzman, 2012). One physiological adjustment that can result from ACEs is a change in the regulation of gene expression (Liu & Nusslock, 2018; Meaney & Szyf, 2005). Research that investigates environmental influences—like ACEs—on gene expression is called Epigenetics. Epigenetics elucidates gene x environment mechanisms—that is, how an environment can get under the skin. This study hypothesized that an epigenetic mechanism may connect ACEs with LoC. Not only are prominent researchers hinting that an epigenetic mechanism exists between ACEs and detrimental outcomes, (Hertzman, 2012; Hertzman & Boyce, 2010; Shonkoff et al., 2012), but recent advances in genetic research also suggest that this is entirely plausible (Galea et al., 2011; Hyman, 2009; Liu & Nusslock, 2018; Meaney & Szyf, 2005; Szyf, 2011; Thayer & Kuzawa, 2011).

**Epigenetics and Epigenetic Age**

There are various ways that the expression of genes can be regulated. Epigenetics refers to the several ways that gene expression is regulated not through the alteration of the sequence of nucleotides themselves (i.e., a mutation), but rather modifications to the structure of DNA which result in changes to how genes are transcribed. There are various types of epigenetic
modifications (e.g., DNA methylation, histone modification). The most studied type of epigenetic mechanism is DNA methylation (DNAm) because, in part, DNA methylation is a simpler epigenetic mechanism to study than, say, histone modifications (Rodríguez-Rodero et al., 2010). DNA methylation changes the expression of genes by adding or removing methyl groups to a cytosine-guanine pair (CpG) (Horvath, 2013). In general, a gene is silenced (i.e., switched “off”) as its level of DNAm increases (Moore et al., 2013).

Patterns of DNAm change predictably with chronological age (Rodríguez-Rodero et al., 2010) so much so that “DNA Methylation Clocks” have been developed. A DNAm clock uses DNAm patterns throughout a person’s genome to predict chronological age. While other exist (e.g., Hannum, 2013; McEwen et al., 2020), the most popular DNA methylation clock—in part because it is a freely available online tool—is called the Horvath Clock and predicts with high accuracy chronological age by assessing methylation patterns at 353 specific CpGs (Horvath, 2013). Variability exists between a person’s actual chronological age and the Horvath Clock predicted “epigenetic age”—also called “DNAm Age,” or “biological age.” The difference between a person’s epigenetic age and actual chronological age is called epigenetic age acceleration. Epigenetic age acceleration is negative when the predicted epigenetic age is less than the chronological age and epigenetic age acceleration is positive when the predicted epigenetic age is greater than the chronological age.

Exploring the ACE-Epigenetic Age Link

Evidence suggests that one way ACEs can get under the skin—and influence things like LoC—is through changes to DNAm patterns (Szyf, 2011; Szyf & Bick, 2013). Since ACEs are associated with changes in DNAm patterns, it is plausible to hypothesize that ACEs predict changes in epigenetic age (Fiorito et al., 2017; Hoare et al., 2020; Jovanovic et al., 2017; Sumner
et al., 2019). Further, changes in epigenetic age are associated with not only a variety of detrimental outcomes like obesity (Nevalainen et al., 2017) and smoking (Wu et al., 2019), but also all-cause mortality (Marioni et al., 2015). Moreover, in children, associations between epigenetic age and parameters of development (Simpkin et al., 2017; Suarez et al., 2018) and internalizing behavior (Tollenaar et al., 2021) have been reported. Since changes in epigenetic age are associated with detrimental outcomes, it is conceivable that epigenetic age predicts an external LoC. The model for the potential mechanism connecting ACEs with LoC becomes clear (Figure 1). To summarize and simplify: Adverse childhood experiences may be associated with LoC. Adverse childhood experiences can also modify patterns of DNAm (epigenetic age). Epigenetic age may influence LoC and thus epigenetic age may mediate the association between ACEs and LoC.

**Figure 1**

*Do ACEs influence LoC through changes in epigenetic age?*
Purpose of the Study

Given previous research, ACEs can “get under the skin” and undermine health and wellbeing through changes to epigenetic patterns. The purpose of this research was to evaluate epigenetic age as a mechanism connecting ACEs with LoC: I hypothesized that epigenetic age mediated the relationship between ACEs and LoC in childhood.

Empirical Contributions, Practical Contributions, and Implications of the Study

Previous research empirically supports the associations between the variables in the model (Figure 1: path a, path b, and path c). Specifically, ACEs are associated with LoC—path c (Ahlin & Lobo Antunes, 2015; Hovens et al., 2016; Roazzi et al., 2016) and ACEs are also associated epigenetic age—path a (Han et al., 2018; Jovanovic et al., 2017; Lawn et al., 2018; Sumner et al., 2019). Although evidence is scant, epigenetic age may be associated with facets of cognition—path b (Suarez et al., 2018; Tollenaar et al., 2021). Lastly, there exists no empirically supported biological mechanism explaining the relationship between ACEs and LoC—path c’.

The relationship between epigenetic age and LoC (path b) has yet to be formally investigated and constitutes the first scientific contribution of this study. Moreover, and perhaps more importantly, the combined influence of ACEs and epigenetic age on LoC (path c’) has yet to be formally evaluated and constitutes the second significant scientific contribution of this study. In other words, this study contributes and extends extant literature because it evaluates whether the relationship between epigenetic age and LoC is substantial enough to account for the relationship between ACEs and LoC and act as a biological mechanism (i.e., evaluate epigenetic age as a biological mechanism connecting ACEs and LoC). This second contribution, as it is the thesis of this study, is discussed in detail next.
Most research that reports associations between ACEs and LoC offer no mechanism, biological or not. That is, the extent of the discussion is that the relationship between ACEs and LoC exists and there is no mention of why or how ACEs influence LoC. For instance, after reporting that parenting styles—like in-home supervision and harsh parenting—were found to be related with a child’s LoC, Ahlin and Lobo Antunes (2015) offer no reason why these parenting styles were associated with LoC. Or consider that Pedron et al. (2021)—in a report evaluating the potential of LoC to mediate the relationship between SES and adult health outcomes—note that SES was associated with shifts in LoC but make no mention of how SES changes LoC.

Exceptions exist, however, and reports offer suggestions as to why ACEs may influence LoC. For example, Shifrer (2019), offer a mechanism that involves parent-child socialization. They suggest that the low frequency and low quality of parental socialization with children in low SES families as a possible mechanism influencing a child’s LoC. Or consider that Roazzi et al. (2016), after reporting that ACEs predicted an external LoC in adolescence, posit that the ACE-LoC relationship observed was due to an altered sense of self and an adjustment to mental models regarding how the world works. In other words, the suggested mechanism connecting ACEs and LoC involved changes to thinking.

To sum, largely absent from literature are studies that directly examine potential mechanisms connecting ACEs and LoC—Shifrer (2019) being the lone exception. The suggested mechanisms that appear in literature seem to be included as due diligence on the author’s part. That is, after reporting that ACEs influence LoC, the author is expected to posit a potential explanation as part of their discussion. The changes to mental models and sense of self mechanisms described previously are examples of such a situation. With this in mind, this
research empirically contributed to literature because exploring a potential biological mechanism connecting ACEs and LoC was the primary objective.

Moreover, with regard to the practical importance of this research, I argue that, while suggested ACE-LoC mechanisms—like the changes to sense of self—further the understanding of the ACE-LoC link, they are practically unhelpful because they posit that a change to a facet of cognition (i.e., LoC) is due to changes in another facet of cognition (i.e., changes to sense of self and mental models). Epigenetic age, however, may prove to be a practically relevant biological mechanism linking ACEs and LoC. If there is evidence that epigenetic age is a mechanism connecting ACEs and LoC, it can be used as a tool to assess a problematic developmental trajectory early. An early indicator of problems yet to manifest is a powerful tool and could result in the immediate implementation of an intervention to preemptively ameliorate any problematic characteristics. In other words, efforts to reduce changes to epigenetic age may serve as useful interventions to mitigate the influences of ACEs on LoC.
CHAPTER TWO
REVIEW OF LITERATURE

A vast body of separate literatures exist that discuss ACEs, epigenetic age, and LoC. The objective of the following chapter is twofold. First, give an overview of each variable. The overviews will not be exhaustive, but rather provide the reader with a working knowledge of each variable with special emphasis on how it adds to the model. The second objective is to justify the model by highlighting the relationships, or lack thereof, between ACEs, epigenetic age, and LoC. To this end, chapter two begins with a discussion of ACEs. It continues with a discussion examining the influence of ACEs on LoC. The final portion of chapter two describes the potential for the mediating role of epigenetic age.

What Constitutes an Adverse Childhood Experience?

Describing an ACE runs the following risk: On one hand, allowing and accepting anything that is bad, stressful, or traumatic as an ACE risks tempering or diluting the construct. A dilute construct leads to vague associations and weak empirical research (Portwood et al., 2021). On the other hand, being too stringent with the definition of an ACE leads to sterile and unrealistic associations that lack generalizability. In other words, an ACE definition with too many stipulations fails to capture the depth, breadth, and texture of ACEs. The next sections will examine the spectrum of ACE definitions by describing two types of ACEs—conventional ACEs and expanded ACEs—and argue that poverty is an expanded ACE.

Conventional ACEs: The Original ACEs Study

A natural starting point for a discussion of what is and is not an ACE is the study that coined the term: The Adverse Childhood Experience (ACE) Study (Felitti et al., 1998). Felitti et al. (1998) published the first report that connected ACEs with health risk behaviors and disease.
The ACE Study operationalized adversity into seven types and grouped them into two categories: abuse category (psychological, physical, sexual) and household dysfunction category (substance abuse, mental illness, domestic violence, criminal behavior). The ACEs explored in the original ACEs study have become known as “conventional ACEs.” While a vast body of literature has been produced with the original ACE study as a model and the original ACEs Questionnaire as a tool, a weakness of the original ACE study was that it was conservative with the inclusion criteria of what constitutes an ACE and only included variables within the home and involving a family member (Karatekin & Hill, 2018; McEwen & Gregerson, 2019). It was concluded that the conservative, “domestic” properties of the original ACEs Questionnaire could potentially create a limited definition and victimization profile. This realization led others to expand upon the conventional ACEs and investigate and operationalize adversity outside of a domestic setting.

**Expanded ACEs: Adversity Outside the Home**

The narrow ACE conceptualization and homogeneous population of the original ACEs study—the sample was educated and mostly white (Felitti et al., 1998)—encouraged the development various other ACEs surveys aimed at investigating ACEs outside the home and in heterogeneous populations. For example, the goal of The Juvenile Victimization Scale (JVS) (Finkelhor et al., 2005) was to increase the developmental breadth and comprehensiveness of the available ACE surveys and assess five areas of concern: (a) child maltreatment, (b) conventional crime, (c) sibling/peer victimization, (d) witnessing and indirect victimization, and (e) sexual victimization. The JVS not only retained the conventional ACEs from the original ACEs Questionnaire, but also included items assessing crime and peer victimization. In other words, the JVS expanded upon the conventional ACEs by including experiences outside of the home.
This technique—that of expanding upon the original ACEs Questionnaire and including “outside the home” adversity—is common and various other surveys have been developed in this fashion (Cronholm et al., 2015; Finkelhor et al., 2013; Karatekin & Hill, 2018; Mersky et al., 2017). ACEs that constitute adversity outside the home are described as “expanded ACEs.”

While the use of conventional ACEs has been paramount in elucidating the connection between early experiences and problems with health and wellness later in life, the justification for including expanded ACEs in analyses, as eloquently described by Mersky et al. (2017), are numerous. First, expanded ACEs improve cross-cultural and ecological validity because many ACEs occur outside the home and some ACEs are unevenly clustered within different demographics. Further, factor analysis has shown that expanded ACEs assess different categories of ACEs than the original child abuse and family dysfunction categories inherent within conventional ACE assessments (Mersky et al., 2017).

**Poverty as an Expanded ACE**

While the case for the use of expanded ACEs has been made, there is some debate about whether poverty should be included as an ACE. On one hand, some groups advocate the use of indicators of poverty—like measures of financial difficulty, parental education, or parental employment status—to assess the prevalence of ACEs through outright acknowledgement—that is, declaring that poverty is an ACE (Hughes & Tucker, 2018; Mersky et al., 2017). Others endorse poverty as an ACE by including indicators of poverty or economic hardship in their analysis. For example, Grummitt et al. (2021) include measures of economic hardship as an ACE, along with other conventional ACEs, in one of the most comprehensive meta-analyses regarding ACES and mortality to date. Or consider Appleton et al. (2017), in a meta-analysis
reporting the association between ACEs and cardiovascular disease, report that about one third of
the included studies assesses SES as an ACE.

On the other hand, other groups treat ACEs and poverty as separate phenomena. Indeed, the same cohort in this research (i.e., ALSPAC) was analyzed and poverty was treated as a confounding variable that caused or modified ACEs rather than an ACE itself (Houtepen et al., 2020). The analysis of various other groups mimic Houtepen et al. (2020): When trying to study specific ACEs, or just the conventional ACEs, it seems common to regard poverty and SES as contextual and thus control for their influence (e.g., Flaherty et al., 2006; Schilling et al., 2007). With conflicting perspectives and considering that this research utilized measures of poverty as a predictor, a further discussion regarding poverty as an ACE is warranted.

**Poverty is Considered an ACE.** While excluding poverty as an ACE is defensible—some groups want to capture specific ACE events and remove the influence of poverty—this research embraced indicators of poverty as ACEs for several reasons. First, poverty and other ACEs are likely comorbid and highly correlated (Evans & Kim, 2013; Walsh et al., 2019), and other ACEs occur more frequently in impoverished populations (Anda et al., 2010). That poverty and other ACEs are comorbid and highly correlated is particularly true within the ALSPAC cohort (Lacey et al., 2020). Second, indicators of poverty—when included in models assessing ACEs and detrimental outcomes—improve the degree of prediction of detrimental outcomes (Finkelhor et al., 2015). Last, there is evidence of a causal relationship between poverty and other ACEs (Walsh et al., 2019). In other words, adversity may be rooted in poverty—if poverty exists, ACEs are also likely to exist.

As the number of ACE types increases—that is, assessing conventional ACEs and expanded ACE—including poverty—the number of children experiencing ACEs increases. Thus,
a true assessment of the prevalence of ACEs within a population is difficult because it depends largely on how ACEs are described within the methodological context of specific studies. With that noted however, many children experience ACEs. The prevalence of ACEs is discussed next.

**Prevalence of ACEs**

In short, many kids experience ACEs. Evaluating the prevalence, however, of ACEs is problematic for several methodological reasons. Describing the methodological problems of determining the true prevalence of ACEs serves to not only demonstrate that many kids face ACEs, but also highlights the necessary considerations while reviewing literature reporting them.

**Methodological Considerations When Assessing ACE Prevalence**

First, the prevalence of ACEs depends on what each study defined and included as an ACE. For example, Feletti et al. (1998)—which is wave I of the original ACEs study—report that 47.9% of the sample experienced at least one ACE. By contrast, Merrick et al. (2017) utilized data from wave II of the original ACE study and report that 82.9% of participants experienced at least one ACE. Participant demographics were the same for wave I and wave II. The reason for the sizable difference in ACE exposure—despite the same sample—was the inclusion of a single item in the analysis that assessed spanking. It can be inferred, then, that the increase in ACE prevalence was a result of the inclusion of the additional item rather than an increase in the actual incidence of ACEs. This example highlights the caution needed for interpreting ACE prevalence rates because what is counted as an ACE varies between studies.

As the previous example demonstrates, adding or removing items that widen or restrict the ACE inclusion criteria changes the prevalence of ACEs within a population. Some studies, however, add entire types of ACEs to their surveys. As discussed already, a weakness of the original ACEs study was the conservative definition of an ACE that resulted in the conventional
ACE designation: child abuse and family dysfunction. Several groups—those the advocate the use of an expanded ACEs framework—acknowledge that ACEs can, and do, occur outside the home, and have created types of ACEs that addresses these experiences. For example, along with the conventional ACEs, Cronholm et al. (2015) explored expanded ACEs including items assessing (the child) witnessing violence, feeling discrimination, living in an unsafe neighborhood, experiencing bullying, and living in foster care. As expected, Cronholm et al. (2015) report a higher prevalence of ACEs: 82.8% of the sample report experiencing at least one ACE.

The same study demonstrates another reason why assessing the prevalence of ACEs is difficult. Not only did Cronholm et al. (2015) extend the criteria of what constitutes and ACE and include expanded ACEs, but they also sampled a socioeconomically and racially diverse population. Specifically, compared to the sample population in the original ACE study, Cronholm et al. (2015) reported that participants were more racially diverse, less educated, and younger. As expected, including a more diverse population resulted in a greater prevalence of participants experiencing multiple conventional ACEs: 47.6% of participants reported experiencing 1-3 ACEs and 20.7% reported experiencing at least four (Cronholm et al., 2015). It bears repeating that who is sampled is also an important consideration when investigating the prevalence of ACEs.

As an extension of the previous point, and as another example, consider that Flaherty et al. (2013) reported that more than 90% of children experienced at least one ACE within their sample. Who their sample was, however, was important. Flaherty et al. (2013) studied ACEs within a sample of children being investigated by CPS. Thus, almost by default, each child in the study had experienced at least one ACE. Not only are sample demographics important to
consider—as illustrated by Cronholm et al. (2015)—but also the intentions of the authors to study different groups—representative samples or predefined groups (i.e., children in the CPS system).

A last consideration, and a potential problem when assessing the prevalence of ACEs, is when participants are assessed. On one hand, many inquiries assess the prevalence of ACEs retrospectively and cross-sectionally in adult populations. For example, the original ACEs study (Felitti et al., 1998) surveyed adults about their childhood experiences. This method of assessing ACEs may be problematic because retrospective reports of ACEs may be biased (Green et al., 2010; Hardt & Rutter, 2004). On the other hand, other inquiries utilize a prospective longitudinal design and assesses ACEs throughout childhood (e.g., Houtepen et al. 2020). A prospective design, however, is not free of bias (White et al., 1998). For example, questionnaires regarding childhood abuse may be answered by a parent or guardian, who may be a potential source of abuse. While both methods of assessing the prevalence of ACEs have their merits and both have substantially contributed to our understanding of the influence of ACEs on disease (Anda et al., 2010), assessing ACEs as they occur—that is prospectively and/or longitudinally—may yield truer ACEs prevalence estimates (Widom et al., 2004).

To sum thus far: What constitutes an ACE, who is being sampled, study intentions (i.e., study a representative group or predefined group), and when participants are assessed are important considerations when evaluating the prevalence of ACEs. With the considerations for determining the prevalence of ACEs noted, however, when surveying the literature, the picture of ACE prevalence becomes clear: Most children experience ACEs and many experience multiple ACEs. The next section will summarize the prevalence of children experiencing one ACE and is followed by a section summarizing the prevalence of children experiencing multiple
ACEs. Last is a discussion regarding the prevalence of ACEs within the ALSPAC cohort—the sample from which the data in this research was derived.

**Prevalence of Children Experiencing At Least One ACE**

The prevalence children experiencing at least one ACE is high. Hughes et al. (2017) reported in a meta-analysis analyzing retrospective self-reported data on conventional ACEs, that out of a cumulative sample of 252,467 international participants, 57% had experienced at least one ACE. Worth noting is that the meta-analysis excluded at risk and clinical samples. Table 1 highlights studies absent from this meta-analysis that report similar findings. The trend is clear: Children experiencing an ACE is the norm.

While many children experience at least one type of ACE, an unfortunate characteristic of ACEs is that the adverse experiences are comorbid. In other words, a child that is physically abused is likely to experience a second type of ACE, like emotional neglect. The subsequent discussion will not only highlight the prevalence of children experiencing multiple ACEs, but also make the previous point—that ACEs coexist—more salient.
Table 1

Prevalence of at least one ACE in various studies

<table>
<thead>
<tr>
<th>Citation</th>
<th>Sample Size</th>
<th>% Of people experiencing at least one ACE</th>
<th>ACEs Assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anda et al., (1999)</td>
<td>9,215</td>
<td>63%</td>
<td>Conventional</td>
</tr>
<tr>
<td>Cronholm et al., (2015)</td>
<td>1,784</td>
<td>72.9%</td>
<td>Conventional</td>
</tr>
<tr>
<td>Cronholm et al., (2015)</td>
<td>1,784</td>
<td>63.4%</td>
<td>Expanded</td>
</tr>
<tr>
<td>Dube et al., (2003a)</td>
<td>8,613</td>
<td>68.7%</td>
<td>Conventional</td>
</tr>
<tr>
<td>Edwards et al., (2003)</td>
<td>8,667</td>
<td>43%</td>
<td>Conventional</td>
</tr>
<tr>
<td>Gilbert et al., (2015)</td>
<td>53,998</td>
<td>59.4%</td>
<td>Conventional</td>
</tr>
<tr>
<td>Houtepen et al., (2018)</td>
<td>3,598</td>
<td>77.7%</td>
<td>Conventional</td>
</tr>
<tr>
<td>Merrick et al., (2017)</td>
<td>7,465</td>
<td>82.9%</td>
<td>Conventional and spanking</td>
</tr>
</tbody>
</table>

*Samples reported in the same study*

Prevalence of Children Experiencing Multiple ACEs

As previously discussed, the participants in the original ACEs study did not represent the population of US residents, but rather a population that was white, educated, and middle class (Felitti et al., 1998). In this affluent population, the original ACEs study report that 27% of the 8,056 participants experienced at least two ACEs. In other words, roughly one quarter of the educated, mostly white people with private health insurance experienced a combination of various types of child abuse and maltreatment. Further, about 6% of the sample experienced at least four ACEs ($N = 545$).
Gilbert et al. (2015) found a slightly higher prevalence of multiple ACEs in a similar population. In a sample that was mostly white (80.4%), older (90% older than 35), educated (71% graduated high school), and relatively wealthy (67% making at least $35,000 a year), Gilbert et al. (2015) reported that 15.3% of 53,998 participants experienced at least four ACEs.

Drawing from a younger population as final example, Duke et al. (2010) surveyed 136,549 high school students in Minnesota. Within the surveyed students, 23.8% received free and reduced lunch. For comparison in 2010, 48.1% of students in the US were eligible for free and reduced lunch that same year (National Center for Education Statistics). This population was also 73.2% white and about half lived in a suburban or rural setting. Further, 63% of students surveyed lived with both biological parents. In this arguably affluent population of children, Duke et al. (2010) reported that 14.9% of students experienced at least two conventional ACEs.

To sum thus far, approximately one-of-five children of mostly white and moderately wealthy populations experience multiple ACEs. The prevalence of multiple ACEs in populations of underprivileged children (i.e., minorities or identified as at risk) is higher. The following studies are exemplars of this point.

The Fragile Families Study is comprised of children from unwed mothers and are more likely African American. Jimenez et al. (2016) considered the sample “borderline” at risk and reported that 28% of the children experienced at least two conventional ACEs by Kindergarten. Roughly double the prevalence of at least two ACEs experienced were reported using data from the Chicago Longitudinal Study (93% African American, 7% Hispanic). In this reportedly underprivileged population \((N = 1,142)\), 47.9% experienced at least two conventional and expanded ACEs and 8.3% experience at least five (Merskey et al., 2013). A similar investigation (i.e., 97.4% minority, urban population) was conducted by Burke et al., (2011) who reported
comparable results: 36.5% of the sample of children had experienced at least two conventional ACEs.

Using a broad definition of at risk, The Longitudinal Studies of Child Abuse and Neglect study gathered ACE data on 1,041 at risk children at ages four and six. At risk, in this study, included factors like low-income families, children at a high risk of HIV infection, children placed in foster care, children identified by CPS, and children with young mothers or that were single parents (Flaherty et al., 2006). Roughly one third, 38%, of children had experienced at least two conventional ACEs and 6.3% experienced at least four (Flaherty et al., 2006).

While far from the norm, Clarkson Freeman (2014) reported the prevalence of conventional ACEs for an extremely at-risk population of children—those within a family being investigated by Child Protection Services. In other words, they were selected for the study because they had at least one ACE and thus the CPS investigation. Among this population, during Wave I data collection, 86.5% had at least two ACEs, 70% experienced at least three, and 42% had at least four (Clarkson Freeman, 2014). At Wave II the percentage of children experiencing multiple ACEs increased: 92.3% had experienced at least two ACEs and 50.5% had experienced at least four (Kerker et al., 2015). The conclusion to draw from this study, and the studies in the preceding discussion, is that at-risk children who have one ACEs are likely to experience others.

To conclude and summarize reporting the prevalence of multiple ACEs, in a meta-analysis—which include several studies discussed—Liming & Grube (2018) report that the range of children experiencing at least three ACEs was 12.3% to 70%. Finally, Hughes et al. (2017) report in a meta-analysis that studies report between a 1% and 38% prevalence rate for children experiencing at least four ACEs.


**Prevalence of ACEs Within ALSPAC**

While a full description of the ALSPAC sample is found in Chapter 3, it is appropriate to describe the prevalence of ACEs in the sample used for the current study. The ALSPAC longitudinal cohort is a widely used data set from which to work and numerous studies have documented and reported the prevalence of ACEs (Table 2). While one may think that deriving ACE variables from the same cohort data set would yield the same information regarding the prevalence of ACEs, it does not. To echo the remarks made regarding the problems assessing the prevalence of ACEs: The prevalence of ACEs within the ALSPAC cohort depends on what ACEs are included in the analysis, who is the analytic sample, and when (i.e., what time point) they were assessed.

Table 2 summarizes studies using the ALSPAC cohort data. Roughly 40%-50% of participants within the ALSPAC cohort experienced at least one conventional ACE and Houtepen et al. (2018) report that about 80% of participants report at least one ACE when the assessment included expanded ACEs (e.g., including financial difficulties and satisfaction with neighborhood). These findings are consistent with the prevalence of ACEs from other large cohorts, many of which were described in the preceding section (e.g., Cronholm et. al., 2015; Edwards et al., 2003; Gilbert et al., 2015). Further, worth noting is a reiteration regarding a trend in the prevalence of ACEs: Within the ALSPAC cohort, as the number of included ACEs increases (e.g., conventional vs. expanded ACEs, or both), the prevalence of children experiencing an ACE—or multiple—increases.
Table 2

Prevalence of ACEs within the ALSPAC cohort

<table>
<thead>
<tr>
<th>Citation</th>
<th>Sample Size</th>
<th>Types of ACEs</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacey et al., (2020)</td>
<td>4,935</td>
<td>Conventional</td>
<td>34.5% 0, 65.5% at least 1, 35.3% at least 2</td>
</tr>
<tr>
<td>Houtepen et al., (2018)</td>
<td>8,021</td>
<td>Conventional</td>
<td>42.4% 0, 57.6% at least 1, 28.65% at least 2</td>
</tr>
<tr>
<td>Soares et al., (2018)</td>
<td>4,444</td>
<td>Conventional</td>
<td>50.4% 0, 49.6% at least 1, 16.4% at least 2</td>
</tr>
<tr>
<td>Tang et al., (2020)</td>
<td>974</td>
<td>Conventional and bullying</td>
<td>Male: 22% 0, 25.9% at least 1, 52.1% at least 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female: 21.5% 0, 24.4% at least 1, 54.1% at least 2</td>
</tr>
<tr>
<td>Houtepen et al., (2020)</td>
<td>9,959</td>
<td>Conventional and bullying</td>
<td>16% 0, 84% at least 1, 60.3% at least 2, 24% at least 4</td>
</tr>
<tr>
<td>Houtepen et al., (2018)a</td>
<td>3,598</td>
<td>Conventional</td>
<td>22.3% 0, 49% at least 2, 13.9% at least 4</td>
</tr>
<tr>
<td>Houtepen et al., (2018)a</td>
<td>1,109</td>
<td>Expanded</td>
<td>74.6% at least 2, 48.6% at least 3, 12.4% at least 6</td>
</tr>
</tbody>
</table>

*a Samples reported in the same study
Regardless of how a study describes ACEs, and regardless of the population under investigation, many children experience ACEs, including children within the ALSPAC cohort. Therefore, ACEs—and their association with outcomes that undermine health and wellbeing—are important to study. The next sections describe the associations of ACEs with physical, behavioral, and cognitive outcomes with special emphasis on one specific aspect of cognition: locus of control.

**Adverse Childhood Experiences and Detrimental Outcomes**

Adverse childhood experiences are important to study because they are associated with outcomes that undermine health, well-being, and cognition. Indeed, the objective of the original ACEs study was to investigate the relationship between child maltreatment and family dysfunction with leading causes of mortality (Felitti et al., 1998). Just as there are multiple types of ACEs (e.g., child maltreatment, family dysfunction), there are multiple categories of outcomes associated with ACEs. Further, and again mirroring the ACEs themselves, the outcomes overlap, have significant correlations between each other, and are comorbid. While there are various ways to group outcomes (e.g., severity, timing, location), the next three sections will describe three *types* of outcomes related to ACEs: behavioral, physical, and cognitive.

**ACEs and Behavioral Outcomes**

While risky behavior has a myriad of associations, this much is clear: Someone who experiences ACEs is more likely to smoke, drink, do drugs, and engage in risky sexual behavior than someone who does not experience ACEs. Evidence for this relationship is abundant. For example, adults who experienced ACEs where more likely to have multiple sex partners, have STDs, have sex earlier, and be pregnant as a teenager. (Dube et al., 2003b; Hughes et al., 2017;
Wade et al., 2016). Hillis et al. (2004)—using data from the original ACEs study—report similar results and found that the number of ACEs had a direct relationship with a teenage pregnancy.

Similarly, adults that abuse drugs, alcohol, or smoke tobacco were more likely to experience ACEs (Anda et al., 1999; Dube et al., 2003a; Dube et al., 2003b; Felitti et al., 1998; Hughes et al., 2017; Merrick et al., 2017; Mersky et al., 2013; Wade et al., 2016). Not surprisingly, ACEs also predict earlier alcohol (Rothman et al., 2008) and drug use initiation (Dube et al., 2003a).

The trend is the same for alcohol, tobacco, and drug use in children as it is in adults. ACEs were associated with drug use within a sample of high school seniors (Schilling et al., 2007) and Dube et al. (2006) reported that ACEs are associated with drinking alcohol before the age of 14 and increased the likelihood of drinking at all between the ages of 15-17. Interestingly, Dube et al. (2006) conclude that ACEs and alcohol use show a dose-dependent relationship: The greater the number of ACEs, the greater the use of alcohol as a teenager.

Within the ALSPAC cohort—the cohort utilized for this study—Houtepen et al. (2020) assessed 17-year-old participants regarding their risky behaviors and reported associations between ACEs and smoking, illicit drug use, and alcohol use. Specifically, teenagers who had experienced four or more ACEs, compared to children who experience zero ACEs, were roughly three times more likely to use illicit drugs and roughly two times more likely to smoke (Houtepen et al., 2020). Interestingly, the odds of using drugs and smoking within the ALSPAC cohort are less than the values reported in a meta-analysis which reported that children who experience multiple ACEs are about five times more likely to use illicit drugs and about three times more likely to smoke (Hughes et al., 2017). The difference perhaps is accounted for by the fact that the meta-analysis includes adult retrospective reports while the ALSPAC surveyed 17-
year-old participants. In other words, while teenagers who experience ACEs are at risk for drug and alcohol problems, the risk remains pertinent as they transition into adulthood—they can still start to use drugs and alcohol as they age.

Problematic behaviors, such as excess alcohol consumption and tobacco use, are known to be associated with chronic illnesses like cancer (Praud et al., 2016; Sasco et al., 2004) and cardiovascular disease (Pittilo, 2000). Adult outcomes associated with ACEs are intimately interrelated and comorbidities are common. The problematic behaviors discussed above are thought to be mechanistic pathways connecting ACEs with another group of outcomes—physical wellbeing—which is discussed next.

**ACEs and Physical Outcomes**

Adults who report ACEs are less physically healthy (Wegman & Stetler, 2009). For example, in a 32-year prospective study, Danese et al. (2009) report that children who experienced social isolation, maltreatment, and low SES were more likely to be overweight and have other concerning metabolic risk factors (e.g., high glycated hemoglobin, high total cholesterol, high low-density cholesterol, high blood pressure). Along the same lines, the link between ACEs and BMI (Burke et al., 2011) and metabolic dysfunction (Tamayo et al., 2010) were also found to be strong. The link between adversity in childhood and the leading cause of death in the United States—cardiovascular disease (CVD)—is very strong and serves as an important exemplar emphasizing the reason that ACEs are important to study.

Cardiovascular disease is strongly associated with ACEs (Appleton et al., 2017; Su et al., 2015; Wade et al., 2016) and the association between ACEs and CVD is robust. As evidence, consider that Appleton et al. (2017) report that, despite the heterogeneity in how ACEs are assessed (i.e, when, who, and what), the relationship between ACEs and CVD is often dose
dependent. Moreover, in one meta-analysis, 8-12% of all cases of CVD were found to be attributed to ACEs. In other words, had the ACEs not occurred, an 8-12% drop in CVD outcomes would result. This translates to roughly 219,000 annul deaths (Grummitt et al., 2021). Lastly, the relationship between ACEs and CVD is so consistent that CVD risk factors (e.g., elevated triglycerides, hypertension, waist circumference) have been identified as biomarkers for adversity in childhood (Deighton et al., 2018).

Along with cardiovascular disease, Felitti et al. (1998) noted associations between conventional ACEs and other leading causes of death like lung disease, skeletal fractures, liver disease, obesity, and cancer.

Liver and lung disease may increase as a function of alcohol and tobacco use—noted earlier for their association with ACEs—but the increase incidence of cancer is harder to explain. The finding that cancer in adulthood is associated with ACEs, however, was not an anomaly. Holman et al. (2016), for instance, reviewed literature with the sole purpose of investigating the relationship of ACEs and the incidence cancer and concluded that the evidence for the association is substantial. The same study reporting the attributable percentage of CVD outcomes with ACEs (Grummitt et al., 2021) also report that about 82,000 annual cancer death are associated with adversity in childhood. Further, there is evidence that certain types of ACEs are associated with certain types of cancer. For example, females who experienced child sexual abuse was found to be associated with cervical cancer (Coker et al., 2009).

The impacts of ACEs on indicators of physical well-being also manifest long before adulthood and there is evidence that ACEs influence the well-being of teenagers. For example, Schilling et al. (2007) note that ACEs were related to teenagers with health concerns that required a doctor which suggested an association with ACEs and overall poor physical health.
Flaherty et al. (2006) report similar finding in cohorts of elementary-aged children. Further, Burke et al. (2011) reported an association between ACEs and obesity in an urban population of children.

ACEs, Mental Health, and Cognition

The association between ACEs and facets of mental health and cognition mirror those between ACEs and concerning behavior and physical dysfunction. A person who experiences ACEs is more likely to suffer from mental health disorders in adolescence and adulthood. Exemplars as evidence for this association are abundant. For example, Edwards et al. (2003) found that mental health scores were indirectly related to the severity of ACEs: The greater the severity of ACEs, the lower the mental health score (i.e., the more dysregulated). Similarly, in a particularly eye-opening report, Hughes et al. (2017) note a greater probability of experiencing a mental health issue for adults who experienced four or more ACEs. Specifically, a person who reported four or more ACEs were about 3.5 time more likely to suffer from anxiety (OR: 3.70), about 4.5 times more like to suffer from depression (OR: 4.40), be less satisfied with life (OR: 4.36), and over 30 times more likely to attempt suicide (OR: 30.14). Other reports indicate the same concerning associations: Adults who experienced ACEs are more likely to be depressed and attempt suicide (Danese et al., 2009; Dube et al., 2003b; Felitti et al., 1998; Merrick et al., 2017, Wade et al., 2016).

The associations between ACEs and detrimental mental health outcomes in adolescents parallel those observed in adults and the relationship between ACEs and mental health, anxiety, and depression is strong (Spinhoven et al., 2010). For example, surveying recent high school graduates, Schilling et al. (2007) report an association between ACEs and depression. Scott et al., (2010) report similar findings: they prospectively surveyed young adults and report that
ACEs were associated with mood disorders and anxiety. The same relationship between ACEs and outcomes related to mental health is also present within the ALSPAC cohort. Tracey et al. (2019) developed adversity trajectories based on 10 domains of childhood adversity and report that membership within high adversity trajectories were associated with an increased risk of depression in young adulthood. Lastly, consider that internalizing/externalizing behaviors—often comorbid with mood disorders, depression, and anxiety—were reported to be associated with ACEs (Bevilacqua et al., 2021; Henry et al., 2021).

Along with problems surrounding mental health, ACEs are also associated with other features of cognition like ADHD diagnosis (Brown et al., 2017). Burke et al. (2011) report that a child who experienced at least one ACE was roughly 10 times more likely to develop a behavior or learning problem and a child experiencing at least four ACEs were roughly 32 times more likely to develop behavioral or learning problems. The increase in likelihood of developing behavioral or learning problems exemplified by these data demonstrate the potential that ACEs have on influencing cognition. Another facet of cognition known to be associated with ACEs is locus of control, which is discussed next.

**Locus of Control**

Locus of control (LoC) refers to how a person views the rewards, consequences, punishments, and reinforcements of their behavior (Rotter, 1966). An internal LoC is the belief, for example, that rewards are contingent upon one’s own actions. In contrast, an external LoC is the belief that rewards are the result of a source independent from the individual, such as chance, luck, or another powerful person. In other words, LoC refers to how a person assigns causality: Are rewards, punishments, and reinforcements the result of their own behavior or do they occur despite their behavior?
**The Development of Locus of Control**

Locus of control develops in childhood and is shaped by many things. Often cited antecedents of a person’s LoC are parenting styles (i.e., authoritative, permissive, authoritarian) and parenting characteristics like level of involvement and degree of nurture (Carton et al., 2021). For example, within the ALSPAC cohort, Nowicki et al. (2018a) report that children that were emotionally nurtured (e.g., cuddled, read to, brought to engaging activities) possessed a more internal LoC. The same study reported children from homes that had the television on most of the day and children from homes where the mother thought that the pet should be considered part of the family were more likely to report an external LoC.

While lack of emotional nurturance might be considered an ACE in itself, other ACEs are also associated with LoC. In fact, the relationship between ACEs and LoC appears to be strong; it is summarized next.

**ACEs Influence Locus of Control**

ACEs lead to feelings of lack of control. A vast literature supports this proposition. As several exemplars, first consider Roazzi et al. (2016) who reported that compared to controls, maltreated children—which included experiences like physical abuse, sexual abuse, and neglect—exhibited a more external LoC. Harsh parenting was found to be negatively associated with an internal LoC (Ahlin & Lobo Antunes, 2015). Lastly, echoing the report of Roazzi et al. (2016), experiences of emotional neglect, physical abuse, psychological abuse, and sexual abuse were found to be associated with LoC in large sample (N = 2,981) of people in the Netherland Study of Depression and Anxiety (Hovens et al., 2016).
The Implications of Understanding the ACE-LoC Relationship

A person with an external LoC is likely to experience other detrimental issues at the same time. In other words, since ACEs are associated with a myriad of detrimental outcomes, a person with an external LoC is likely to have also have other cognitive (Hovens et al., 2016), physical (Neymotin & Nemzer, 2014), and behavioral issues (Lassi et al., 2019). This trend is especially salient within the ALSPAC cohort. For example, multiple studies have reported an association between ACEs and external LoC—assessed at ages 8 and 16—and psychotic symptoms like hearing voices and believing that they (the child) were being sent special messages (Sullivan et al., 2021; Thompson et al., 2011).

Moreover, because a person has an external LoC, they are more likely to attribute other outcomes related to ACEs—like cardiovascular disease and anxiety—to forces outside of their control—and are thus less likely to seek change or adhere to a treatment regime aimed at ameliorating the disease state. For example, Anastasiou and colleagues (2015) report that people with an external LoC were more likely to lose weight and regain it compared to people with an internal LoC who lost weight and kept it off. This is like a “one-two punch,” ACEs not only influence LoC; but LoC also influences how the person thinks about other detrimental outcomes associated with ACEs.

Not only is LoC influenced by ACEs, but LoC is also a mediating and moderating mechanism connecting ACEs with other outcomes. Indeed, most of the literature reporting the relationship between ACEs and LoC are within this context. For example, Hovens et al. (2016) reported an association between ACEs and LoC while examining the role of different personality characteristics, like LoC, in mediating the influence of ACEs on depressive symptoms. Or consider that Culpin et al. (2015) found within the ALSPAC cohort 34% of the influence of
ACEs (i.e., poverty) on depression in adolescence was explained by an external LoC. Also, within the ALSPAC cohort, Fisher et al. (2013) reported that LoC was an important mediator between harsh parenting and psychotic symptoms in adolescence. Similarly, the association between parenting styles and bully-victim experiences was mediated by LoC in an adolescent population (Georgiou et al., 2017). Worth noting from this report was that authoritarian parenting styles and bully-victim experiences were partially mediated by LoC at school. The context of a school setting brings to light another important concept of ACEs and LoC: the interrelatedness of ACEs, LoC, and academic achievement.

That ACEs influence LoC is especially salient for child and adolescent populations because of the role LoC plays in academic settings. Locus of Control is associated with various school measures like GPA, academic self-concept (Albert & Dahling, 2016), and academic success in general (Findley & Cooper, 1983). Since ACEs influence both LoC and academic success (Bethell et al., 2014; Blodgett & Lanigan, 2018; Evans et al., 2020), LoC is a plausible mechanism linking ACEs with academic success. The interrelatedness of ACEs, LoC, and academic success highlights another reason that understanding the mechanism between ACEs and LoC is important.

While the problems and implications surrounding an external LoC are well documented, and the antecedents of LoC are identified (e.g., parenting style, ACEs), how ACEs influence a person’s LoC is not well understood. That is, through what mechanism are ACEs acting on LoC? I hypothesized that ACEs influence changes in epigenetic patterns and that these epigenetic changes influence LoC.
Mechanisms Connecting ACEs With Detrimental Outcomes

To “get under the skin” and influence a detrimental outcome—like cardiovascular disease, anxiety disorders, or an external LoC—an experience must change the biology of a person. That is, an experience must occur and subsequently become biologically embedded (Hertzman, 1999). There are various branches of research indicating that ACEs do just that—become biologically incorporated. For example, several studies have reported that experiencing ACEs are associated with changes in the volume of the brain regions like the hippocampus and amygdala (Heim et al., 2008; Herzog et al., 2020; Teicher et al., 2012) or dysregulated HPA function (Heim et al., 2008; Morris et al., 2019). Taken together, however, much of the research implies the same thing: ACEs may alter physiology.

Epigenetics

Another physiological change associated with ACEs is changes to gene regulation. How genes are regulated and expressed is different between populations of children that experienced ACEs and those that did not (McGowan et al., 2009).

Epigenetics is the field of science investigating how experience and environment alter gene expression and is a new biological mechanism with the potential to link ACEs with detrimental outcomes. Changes on nucleotides rather than changes within nucleotides characterize epigenetic modifications that result in variation of gene expression. In other words, gene expression through epigenetic mechanism is the result of changes that occur to the structure of DNA—for example how the chromatin is packaged—rather than changes to a sequence of nucleotides, such as a mutation.

The most studied epigenetic modification is DNA methylation (DNAm). DNA methylation modifies the expression of genes by adding or removing a methyl group to a
cytosine-5 guanine (CpG) dinucleotide (Horvath, 2013). In most cases, adding methyl groups to a CpG dinucleotide silences the expression of that DNA sequence (Moore et al., 2013). The silencing of gene expression via the DNA methyl (DNAm) mechanism results in fundamental biological components like embryonic cell differentiation and X chromosome inactivation, to note several notorious examples.

Twin studies reveal that DNAm levels in monozygotic twins are highly correlated at an early age and thus appear to be under genetic influence. Concordance of DNAm levels, however, decrease over time (Lipman & Tiedje, 2006; Wong et al., 2010). This variation in levels and patterns of DNAm are accounted for by epigenetic drift (Martin, 2005) and environmental differences (Lipman & Tiedje, 2006). That DNAm patterns reflect differences in environmental conditions is important because it demonstrates that experience can modify physiology and phenotypes—that is, it shows how experience can become biologically embedded or get under the skin. Moreover, childhood seems to be a sensitive time for environmental conditions to get under the skin and influence physiology and developmental trajectories.

**Childhood as a Sensitive Period for Changes in DNA methylation (DNAm)**

Humans are adaptively plastic; subtle shifts in the environment are met with subtle shifts in phenotypes (Bateson et al., 2014). In other words, an immutable set of genotypes, through the regulation of their expression, can produce a variety of different phenotypes that match an environment (Hochberg et al., 2011). Epigenetic modifications are some of the proposed mechanisms controlling these shifts in phenotype because epigenetic modifications can change the degree to which a single gene, or a suite of genes, are expressed (Heindel et al., 2015; Szyf & Bick, 2013). Thus, an environmental change induces a modification to epigenetic marks which adjusts a phenotype that is better suited for the new environment. While this adaptive plasticity...
may occur throughout a lifetime, maximal plasticity seems to occur in childhood and
development (Hochberg et al., 2011). Further, it seems that childhood and development are
sensitive periods whereby the influence of the environment is particularly salient and epigenetic
marks are most dynamic.

Dunn et al. (2019) is an exemplar demonstrating that childhood may be a sensitive
window for an environment to influence patterns of DNAm. Using the ALSPAC cohort and
comparing the timing of environmental exposures—in this case adversity—with DNAm patterns
(i.e., changes), Dunn et al. (2019) reported that the timing of the adverse exposure accounted for
more of the variability in DNAm patterns than did the amount or recency of the exposure. In
other words, when the adversity was experienced mattered more than how much adversity was
experienced or how recent the adversity was. That childhood and development are characterized
by dynamic shifts in DNAm levels makes this sensitive window an interesting starting place to
investigate the potential for an epigenetic mechanism to connect ACEs and LOC and strengthens
the rationale for this research.

**In Support of the ACE-DNAm Link: EWAS and Candidate Genes**

The work conducted by Dunn et al. (2019) demonstrated that childhood may be a
sensitive window for epigenetic changes as influenced by adversity (i.e., SES), and is an example
of an Epigenome Wide Association Study (EWAS): A method of analysis that evaluates
associations between patterns of DNAm and outcomes. Other EWAS report similar associations
between childhood adversity and significant changes to DNAm patterns (Borghol et al., 2012;
Esposito et al., 2016; Laubach et al., 2019) including other work done with the ALSPAC cohort
(Lussier et al., 2021). These studies demonstrate that adversity is met with changes to DNAm
patterns. Moreover, based on these reports, it seems that changes to DNAm patterns is a viable
explanation describing how childhood adversity may get under the skin. The excitement for the associations between adversity and DNAm patterns, however, needs to be tempered because EWAS report nothing regarding causation and simply that adversity is matched by shifts in DNAm patterns. Further, also existing are reports of null associations between adversity and DNAm (Marzi et al., 2018).

In further support of the childhood adversity-DNAm relationship, candidate gene studies have also identified differential patterns of DNAm levels on individual genes. In other words, childhood adversity is associated with significant changes to DNAm levels on relevant genes—relevant in the sense that the level of DNAm might imply a specific phenotype. For example, various reports indicate that the glucocorticoid receptor gene (NR3C1) is hypermethylated and the FK506 Binding Protein 41 gene (FKBP5) is hypomethylated in people who experienced adversity in childhood (Cicchetti & Handley, 2017; Tyrka et al., 2015; Tyrka et al., 2016). The NR3C1 and FKBP5 genes are considered candidate genes because not only are they exhibit different levels of DNAm patterns in people who experienced adversity, but they are also relevant in that they are related to the stress response and thus may influence a phenotype. Along with EWAS and candidate gene studies, reports using a new method to evaluate DNAm patterns—called epigenetic age—also suggest that childhood adversity can get under the skin through changes the DNAm.

**Epigenetic Age**

Levels and locations of DNAm throughout a genome change not only as a result of a variable environment, but also with the passage of time and chronological age (Rodríguez-Rodero et al., 2010). Since DNAm patterns change with age, they can be used to predict chronological age with high accuracy. Indeed, algorithms have been developed for such a
purpose. So called “DNA methylation clocks” can estimate the chronological age of a person using DNAm patterns. While other exist, for example the Hannum Clock (Hannum et al., 2013), the clock that is the most widely used is called the Horvath Clock (Horvath, 2013, Horvath & Raj, 2018).

To develop the Horvath Clock—and other DNA methylation clocks—a training set of epigenetic data was used to regress levels of DNAm at different CpG locations with chronological age. The CpGs that contributed most to the prediction model were retained—353 CpGs were kept in the Horvath Clock’s case. Each CpG has a coefficient used in the regression equation to predict chronological age. To predict a person’s chronological age, levels of DNAm at the 353 CpGs, called beta values, are entered into the Horvath Clock regression equation (Horvath, 2013). The output represents the persons predicted chronological age based on the CpG regression coefficients and the person’s beta values. In other words, The Horvath Clock uses the level of DNAm at 353 CpG locations to calculate a “DNA methylation age” or “epigenetic age;” which is the predicted chronological age of that specific person based on their DNAm patterns.

While the Horvath Clock is correlated with chronological age with a high degree of accuracy—r = .96 (Horvath, 2013)—there is a difference between a person epigenetic age (i.e., the predicted chronological age), and the person’s actual chronological age. The difference between a person’s chronological age and epigenetic age is called “epigenetic age acceleration.” Epigenetic age acceleration is negative when epigenetic age is less than chronological age and positive when epigenetic age is greater than chronological age.
The Influences and Outcomes of Epigenetic Age

Epigenetic age is important to study for two reasons. First, epigenetic age, since patterns of DNAm are influenced by environmental factors (Lipman & Tiedje, 2006), is also influenced by environmental factors (Horvath & Raj, 2018). Second, an accelerated epigenetic age is related to detrimental outcomes including all-cause mortality (Marioni et al., 2015). These characteristics of epigenetic age are discussed next.

Epigenetic Age is Influenced by Environmental Factors. While reports are conflicting, research indicates that favorable lifestyle exposures, like exercise and vegetable consumption, decrease epigenetic age (Quach et al., 2017). The opposite is also true: Unfavorable lifestyle exposures, like smoking (Dugué et al., 2018), increases epigenetic age. Another lifestyle exposure that influences epigenetic age is adversity in childhood.

Adverse Childhood Experiences Influence Epigenetic Age

Research is beginning to show that certain environmental factors are related to epigenetic age. One such factor is adversity in childhood. When changes to epigenetic age are detectable is an important question. In other words, does the influence of ACEs on epigenetic age manifest in adulthood—or are changes to epigenetic age detectable in childhood?

Adverse Childhood Experiences and Epigenetic Age in Adults. Several studies report that ACEs are related to an accelerated epigenetic age in adults. For instance, Han et al., (2018) found that childhood trauma was associated with an accelerated epigenetic age in adulthood ($N = 811$). Lawn et al. (2018) report something similar within the ALSPAC cohort: Sexual abuse experienced in childhood was associated with an accelerated epigenetic age in adulthood ($N = 989$). With those positive associations noted, however, Wolf et al. (2018) report in a meta-analysis that PTSD and lifetime trauma were not associated with epigenetic age in adulthood.
While results are mixed, it seems that ACEs may influence epigenic age in adulthood. Some studies, however, report that adversity, trauma, and SES at present time—that is, when the data was collected in adulthood—is associated with epigenetic age and measures of childhood adversity and SES were not. For example, Simons et al. (2016) report that financial pressure and lower income—as adults—were related to an accelerated epigenetic age in adulthood but childhood adversity was not (N = 100). Or consider that combat trauma adults (N = 96) was associated with an accelerated epigenetic age but childhood trauma was not (Boks et al., 2015). Other studies report that cumulative life stress and not current or childhood trauma is associated with epigenetic age (Zannas et al., 2015). Even others fail to any relationship between adversity or SES and epigenetic age (McCrory et al., 2019).

To sum, it seems that ACEs—and any adversity at any time—may influence epigenetic age in adulthood. Does the same pattern appear when ACEs and epigenetic age are measured during childhood? In other words, within populations of children, are ACEs associated with epigenic age? Several recent studies suggest that it is.

**Adverse Childhood Experiences and Epigenetic Age in Children.** A smaller literature reports that epigenetic age in childhood is associated with ACEs. Specifically, violence seems to be an important influence on epigenetic age within populations of children. For example, within the ALSPAC cohort, teenage girls that were physically abused, compared to girls who were not, were on average 1.22 epigenetic age years older 95% CI [.06, 2.38] (Tang et al., 2020). Moreover, Jovanovic et al. (2017), in a study surveying 101 children and teenagers (ages 6-13), report that exposure to violence predicted epigenetic age $R^2 = .085$, $p = .003$. Specifically, children who had a one year accelerated epigenetic age had twice as much violence exposure than children without an accelerated epigenetic age. Similarly, Sumner et al. (2019) found that
exposure to the threat of violence predicted epigenetic age within 247 children ages 8-16, $R^2 = .019$, $p < .05$. Interestingly, Sumner and colleagues (2019) also reported a null association between a different form of adversity—experiencing deprivation (i.e., neglect, food insecurity)—and epigenetic age.

Along with violence, emotional abuse influences epigenetic age in children. For instance, emotional abuse in the form of racial discrimination in young adulthood was associated with an accelerated epigenetic age at age 22 in a population of African American adolescents according the Hannum epigenetic clock $b(294) = 1.47$ (years), $p = .020$ (Brody et al., 2016a). Similarly, conventional ACEs, and specifically emotional abuse, were also found to be influential within the ALSPAC cohort and were associated with epigenetic age acceleration in female teenagers. Specifically, compared to girls reporting zero ACEs, girls reporting four or more were a mean epigenetic age difference of 1.65 years older (95% CI: .25, 3.04). Girls that were emotionally abused, compared to girls who were not, were on average 1.20 years older 95% CI [0.015, 2.26] (Tang et al., 2020).

Moreover, Brody et al. (2016b) report that parental depression measured when children were 11 predicted an accelerated epigenetic age at age 20, $b(157) = 1.815$, $p < .05$. Interestingly, in the same study, children who experienced harsh parenting at 11 years old, and whose parents subsequently participated in a parenting intervention called the Strong African American Families (SAAF) program, did not have an accelerated epigenic age at age 20 compared to controls. In other words, the SAAF program accounted for the difference in epigenetic age between children whose parents received the training and children whose parents did not. This last example highlights the potential influence that the environment, particularly ACEs, may
have on epigenetic age. It demonstrates that a favorable influence—parenting styles becoming less harsh and more positive—can have a significant impact on epigenetic age.

Lastly, poverty influences epigenetic age in children. Peng et al. (2019) report a higher epigenetic age in children from mothers with lower levels of education compared to children from mothers with high levels of education ($N = 408$). From the same cohort, children from single mothers had a higher epigenetic age than children from wed mothers (Peng et al., 2019). Also evaluating poverty and economic hardship, Chen et al. (2016) report that when divided into high hardship, downward mobility, and low hardship groups, a significant difference in epigenetic age existed between groups. To paraphrase the authors (Chen et al., 2016): The more time experiencing economic hardship, the greater the epigenetic age $F (2,310) = 4.88, p = .008$.

While the reports from Peng et al., (2019) and Chen et al., (2016) both indicate that poverty is associated with epigenetic age in children, this relationship still requires elucidation because other reports fail to find the same relationship. Interestingly, in a cohort of teenagers ($N=955$), SES was not associated with epigenetic age as calculated by the Horvath Clock but was associated with epigenetic age using the Hannum Clock (Marini et al., 2020). This is an important exemplar demonstrating that not only does the relationship between lifestyle factors and epigenetic age need to be analyzed carefully, but so do the clocks used to evaluate epigenetic age.

To summarize, the influence of ACEs on epigenetic age can be detected in both adulthood and childhood. This is worrisome because changes in epigenetic age are associated with detrimental outcomes.

**Epigenetic Age is Associated With Detrimental Outcomes.** The second reason that epigenetic age is important to study is that changes in epigenetic age are associated with
detrimental outcomes. Various reports indicate that an accelerated epigenetic age is related to increased risk for mortality (Marioni et al., 2015). Epigenetic age is also associated with various other indicators of health and longevity in adult populations. The greater the acceleration of epigenetic age, the more pessimistic the outlook for indicators of cardiovascular disease (Horvath et al., 2016), cancer (Zheng et al., 2016), and Alzheimer’s Disease (Levine et al., 2015).

Epigenetic Age is Associated With Physical, Developmental, and Pubertal Outcomes in Children. Most of the associations between epigenetic age and detrimental outcomes come from samples of adults. The connection, however, between epigenetic age and detrimental outcomes has not been missed by developmental biologists. Indeed, hypotheses regarding probable associations between epigenetic age and parameters of development in populations of children are recently appearing in literature (Belsky, 2019).

On one hand, the findings regarding children and associations with epigenetic age, though limited, seem to parallel that of adult populations: Epigenetic age acceleration is associated with markers indicating poor health. For example, epigenetic age was found to be a predictor of inflammation and BMI in a population of 17-year-old teenagers (Huang et al., 2019).

On the other hand, the associations between epigenetic age and parameters of physical and/or pubertal development are suggestive at best. For example, epigenetic age was found to be associated with certain components of pubertal development like age at menarche (Binder et al., 2018), tanner stage and Pubertal Development Scale (Simpkin et al., 2017; Suarez et al., 2018), but not age at puberty estimated by age at peak height velocity (Simpkin et al., 2017). Further, epigenetic age may demonstrate conflicting influences on factors contributing to development within the same population: Simpkin et al. (2017) report that epigenetic age was positively
associated with BMI and weight but negatively associated with changes in height and fat mass in the same adolescent population. To sum, the scant literature addressing epigenetic age and physical, pubertal, and developmental in outcomes in children weakly implies that an accelerated epigenetic age is associated with markers indicating an accelerated developmental pathway. With weak associations between epigenetic age and physical components of development, hypotheses regarding associations between epigenetic age and other related components of development, like cognition, may be questionable.

**Epigenetic Age and Associations With Facets of Cognition.** While there are reports of epigenetic influences on executive function (Ibrahim et al., 2018; Xu, 2015), reports of epigenetic age and facets of cognition in adults (Chouliaras et al., 2018; Degerman et al., 2017), and literature regarding DNAm of candidate genes and cognition (Chouliaras et al., 2018; Liu et al., 2018), largely absent from literature are discussions addressing the relationship between epigenetic age and facets of cognition in children. One notable exception is a study of African American adolescents ($N = 292$) living in rural Georgia which found that among low SES teenagers, higher self-control—assessed at ages 17-20—predicted an accelerated epigenetic age when participants were 22 years old (Miller et al., 2015). In other words, among low SES African American youth, higher self-control was related to older epigenetic age. The authors concluded that among low SES youth, high self-control was a double-edged sword. While self-control was associated with lower depression, drug use, internalizing, and aggressive behavior, it also predicted a higher epigenetic age. Worth noting is that the reverse trend was true for high SES youth: as degree of self-control increased, epigenetic age acceleration decreased (Miller et al., 2015).
With that exception noted, however, several reports discuss associations between cognitive dysfunction and epigenetic age in children. For example, internalizing behaviors at 2.5 years old predicted an accelerated epigenetic age at 6 years old in a sample of children (Tollenaar et al., 2021). Further, an accelerated epigenetic age at 6 years old predicted internalizing behaviors at ages 6-10 in the same sample. Similarly, Suarez et al. (2018) report that an accelerated epigenetic age was associated with higher odds of internalizing behaviors, withdrawal, affective problems, and symptoms of anxiety/depression, while associations between epigenetic age and aspects of cognition (i.e., estimated intelligence) were found to be null.

**Literature Overview, Current Study, and Hypothesis**

After reviewing the relevant literature that describes ACEs, epigenetic age, and LoC, we can make three conclusions. First, ACEs are associated with LoC. To echo remarks made before: various studies report that—regardless of what and how it was assessed—ACEs are associated with LoC. For example, children that were maltreated (Roazzi et al., 2016), harsh parenting (Ahlin & Lobos Antunes, 2015), and conventional ACEs (Hovens et al., 2016) were all associated with LoC.

Second, ACEs are associated with epigenetic age in childhood. For instance, children that were exposed to violence (Jovanovic et al., 2017; Sumner et. al, 2019) and emotionally abused (Brody et al., 2016a; Brody et al., 2016b; Tang et al., 2020) were reported to have an accelerated epigenetic age.

Last, limited evidence suggests that epigenetic age is associated with facets of cognition in children. For example, self-control (Miller et al., 2015), and internalizing (Suarez et al., 2018; Tollenaar et al., 2021) were found to be associated with epigenetic age.
The chain of association becomes clear: adversity in childhood influences epigenetic age, epigenetic age then influences LoC. Building upon these associations, it is plausible that epigenetic age acts as a mechanism connecting ACEs and LoC. Specifically, this study hypothesized that the relationship between poverty experienced as a child—operationalized as measures of financial difficulties and neighborhood stress—and LoC—assessed by the Children’s Nowicki-Strickland Internal-External scale at age 8—is mediated by epigenetic age as calculated by the Horvath Clock (Figure 1).

**Justification of Financial Difficulties and Neighborhood Stress Independent Variables**

Among the conventional and expanded ACE types, there exist numerous potential candidates for ACE variables. This research, however, used the individual poverty indicators financial difficulties and neighborhood stress as the ACE independent variables—and exclude others (e.g., maternal depression, parental drug abuse)—for several reasons.

First, and perhaps most importantly, financial difficulties and neighborhood stress—compared to other adversity types like maternal psychopathology and emotional abuse—have been shown to predict the greatest number of genome-wide methylation changes in the ALSPAC cohort (Dunn et al., 2019). Thus, this study used financial difficulties and neighborhood stress as adversity measures since epigenetic age represents patterns and levels of DNAm.

Second, indicators of poverty improve the degree of prediction when the relationship between ACEs and detrimental outcomes are assessed (Finkelhor et al., 2015) suggesting that poverty accounts for a substantial portion of the variance in the relationship between ACEs and detrimental outcomes.

Third, poverty is highly associated and comorbid with other ACEs (Anda et al., 2010; Evans & Kim, 2013; Mersky et al., 2017; Walsh et al., 2019). To succinctly summarize this
thought: “Being poor is associated with so many childhood adversities that it may be considered an ACE in itself, more persistent and pervasive than all others.” (Hughes & Tucker, 2018, p. 124). This pattern—that poverty is strongly associated with other ACEs—is particularly pronounced within the ALSPAC cohort. For instance, Lacey et al., (2020) reported that poverty was not only strongly related to clusters of ACEs, but also individual ACEs. In fact, the relationship between poverty and ACEs were so strong that Lacey and colleagues (2020) suggest that reducing childhood poverty as one strategy to reduce ACEs within ALSPAC.

Fourth, poverty may be the root cause of other ACEs. (Evans & Kim, 2013). Walsh and colleagues (2019), in a meta-analysis specifically targeting the relationship between childhood socioeconomic position (SEP) and ACEs, state that the evidence presented in their evaluation suggests that childhood SEP is a determinant of adversity. They continue to note that the longitudinal nature of many of the reviewed studies strengthens the claim that the association between SEP and adversity is causal (Walsh et al., 2019). To sum and rephrase thus far: The use of financial difficulties and neighborhood stress was justified because indicators of poverty are associated with changes to DNAm patterns, poverty may serve as a proxy for adversity experienced in childhood, poverty accounts for a substantial amount of variance in detrimental outcomes, poverty is highly correlated with other ACEs, and poverty may be the cause of other ACEs.

Moreover, the inclusion framework described earlier justifies including financial difficulties and neighborhood stress as ACE independent variables while excluding others; some variables within the data set, though stressful, are not considered ACEs. For example, within the ALSPAC cohort, a variable exists entitled ALSPAC Life Events Score. This variable is an aggregation of items that total the number of “life events” experienced by the child by 42 months
of age. While the variable does include conventional ACES (e.g., child was sexually abused, child was physically abused), it also includes items that fall outside of the conventional and expanded ACE framework (e.g., child’s pet died, child began new childcare). To reiterate, while these may be adverse events for a child, existing literature does not support their inclusion as ACEs. Since the ALSPAC Life Events Score includes extraneous experiences, its inclusion as an ACE in the model is inappropriate and it is thus excluded from the model.

A final reason for including financial difficulties and neighborhood stress in the model and excluding other ACE variables is one of convenience. While the ALSPAC data set may contain other ACE variables, the subset of ALSPAC data available for secondary analysis did not include them.

Other reasons exist for including indicators of poverty in the model beyond the exclusion of other candidate ACE variables. For example, not only do financial difficulties and neighborhood stress accurately indicate the environment of a child, but they also serve as important predictors of LoC orientation. Using financial difficulties and neighborhood stress as predictors is justifiable because among the factors that influence LoC, (i.e., parenting styles, parental engagement), poverty is particularly salient. Julien Rotter—in the original paper introducing the LoC construct—first described associations between LoC and SES (Rotter, 1966). Further, various reports indicate that SES is leading factor in LoC orientation. For example, Wickline et al., (2011) concluded that “the most significant variable associated with LoC in the present study was SES.” (Wickline et al., 2011, p.46). Several other reports parallel this conclusion (Ahlin, 2014; Ahlin & Lobo Antunes, 2015; Mittal & Griskevicius, 2014; Pedron et al., 2021). Poverty is not only a predictor of LoC, but perhaps the most important predictor of LoC.
Moreover, financial difficulties and neighborhood stress are justifiable predictors in the model because chronic rather than acute events are known to influence epigenetic age. For example, obesity and smoking (Horvath & Raj, 2018) are chronic lifestyle conditions that influence epigenetic age. Poverty is also a chronic condition associated with epigenetic age (Austin et al. 2018; Hughes et al. 2018). For example, African American teenagers that experienced economic hardship during their teenage years exhibited an accelerated epigenetic age at age 20 (Chen et al., 2016). A single event of abuse or being bullied a handful of times is unlikely to influence epigenetic age in a meaningful way and the chronicity of the stimuli—in this case indicators of poverty—seems to be a meaningful factor in epigenetic age (Horvath & Raj, 2018).

Lastly, neighborhood stress and financial difficulty were assessed longitudinally. That is, the ACEs utilized in the model were prospectively assessed at multiple timepoints. Evidence suggests that when ACEs are assessed is important and retrospective reports may be biased (Green et al., 2010; Hardt & Rutter, 2004; Reuben et al., 2016). While an ongoing discussion regarding the strengths and weaknesses of both retrospective and prospective methodologies to assess ACEs exists (e.g., Widom et al., 2004), the preferable method for evaluating ACEs may be as they occur.

To conclude, ACEs are prevalent and are related to LoC. The mechanism connecting ACEs to LoC, however, is not known. This research hypothesized that epigenetic age may mediate the relationship between ACEs and LoC. As ACEs, financial difficulties and neighborhood stress are well suited as predictors in this model because not only are they chronic extended ACEs that serve as a proxy for adversity experienced in general, but they were also
assessed longitudinally, are known to be influential factor in the development of LoC, and induce changes to DNAm.

**Research Question**

1) Does epigenetic age mediate the relationship between ACEs and LoC within the ALSPAC cohort?
CHAPTER THREE

METHOD

A secondary data analysis (SDA) was conducted to examine the potential for epigenetic age to mediate the relationship between ACEs and LoC in the ALSPAC cohort. Exploring the model through SDA was justifiable for numerous reasons. Collecting the measures—ACEs and LoC—would prove challenging for a single investigator. Further, assessing these constructs longitudinally at multiple time points adds an additional layer of complexity. Moreover, collecting DNA samples and processing the epigenetic data presents a substantial constraint. Lastly, collecting the data in a large enough sample to detect an effect might also prove prohibitive (Davis-Kean et al., 2015). In short, the data collection logistics required to evaluate the model, for a single investigator, is an obstacle unlikely to be conquered. Thus, an SDA was appropriate to evaluate the model.

With that noted, this research consisted of three steps: (1) justify the use of the ALSPAC data set for secondary analysis (2) using the measures extracted from the ALSPAC cohort, create poverty (i.e., ACE), epigenetic age, and locus of control variables, (3) conduct the mediation analysis to address the research question.

The Avon Longitudinal Study of Parents and Children (ALSPAC): Participants

Data for the study are drawn from the Avon Longitudinal Study of Parents and Children (ALSPAC). The Avon Longitudinal Study of Parents and Children is a prospective, longitudinal study initiated in the early 1990s. The original goal of ALSPAC was to determine the varying degrees of influence that a person’s genetics and environment contribute to disease, overall health, and development. It considers various genetic, epigenetic, and environmental influences on developmental outcomes (Boyd et al., 2013). This study also investigated both environmental
(i.e., ACEs), and epigenetic influences on a facet of development (i.e., LoC). Thus, the research question and the primary source of data match (Johnston, 2014): The ALSPAC study was established to address the very question asked by this research. This is important to note because a primary concern of SDA is that the original data set was not designed to answer the research question (Schlomer & Copp, 2014).

That the ALSPAC cohort data set and research questions matched, however, says nothing about the quality of the data set. A common drawback when conducting secondary data analyses is the risk, since someone else collected the data, that the desired constructs drawn from the primary data source may be measured inadequately, inappropriately, or partially. Secondary data sets are typically characterized as possessing a large breadth of constructs; each construct, however, lacks measurement depth (Donnellan & Lucas, 2013). In other words, secondary data sets have many constructs evaluated with only a handful of items. As the risk relates to this research, a concern for assessing secondary data is that the ACE and LoC constructs—constructs that are inherently complex and multifaceted—would be assessed with only a few items—hardly enough to capture the true nature of the constructs. A notable strength of the ALSPAC cohort is that it contains an astonishing number of constructs (i.e., breadth) and a startling number of items (i.e., depth). The cohort, in its entirety, contains thousands of people and over 80,000 variables. While a full description of the ACE and LoC measures is forthcoming, in short, many items, at multiple timepoints were available to create the variables used in this research. That the ALSPAC cohort contains many variables and the variables included were assessed not only longitudinally, but also thoroughly (i.e., each measure consists of numerous items) demonstrates that the ALSPAC cohort was a quality set of data from which to evaluate the model. Further, the variables utilized in this research are the variables collected. That is, this research did not modify
or adapt existing data to suit the aim of the study. Other than data management and variable creation, the items and measures were used as-is.

To sum, the ALSPAC data set was appropriate for this study because it matched the research question. The ALSPAC data was a quality set of data to analyze because it contains a vast number of variables, thoroughly assesses each variable at multiple time points, and is managed and distributed by professionals at an academic institution.

**Description of ALSPAC Participants**

The cohort is comprised of mothers and their children residing in Avon, Great Britain. Original recruitment included pregnant mothers with expected delivery dates ranging between April 1st, 1991 and December 31st, 1992 (Fraser et al., 2013). A total of 14,541 pregnancies were originally included in the ALSPAC cohort resulting in 14,676 fetuses (Boyd et al., 2013). The pregnancies resulted 14,062 live births. After one year, 13,988 children remained living. Two additional recruitment phases—to increase the cohort size—resulted in an additional 913 children added to the cohort. In the end, the enrolled sample consists of 15,454 pregnancies resulting in 14,901 live-born children alive at 1 year of age (Boyd et al., 2013).

Data collection started when the mothers were eight weeks pregnant and is ongoing for the mothers and children. Questionnaires dominate the data collection process for psychological, demographic, and behavioral variables while physiological and biological data are collected by clinicians and physicians. Please note that the ALSPAC study website contains details of all the data that are available through a fully searchable data dictionary and variable search tool: [http://www.bristol.ac.uk/alspac/researchers/our-data/](http://www.bristol.ac.uk/alspac/researchers/our-data/).

Written informed consent was obtained from all study participants. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research
Ethics Committees. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

**Final Analytic Sample: Accessible Resource for Integrated Epigenomic Studies (ARIES)**

The final analytic sample used for this study is a representative sub-sample of the 14,901 ALSPAC children—called the Accessible Resource for Integrated Epigenomic Studies (ARIES)—for whom epigenetic data has been collected. From the original 14,901 children, a total of 1,018 were selected based on availability of epigenetic samples. In other words, the 1,018 children included in the ARIES sub-sample have epigenetic data available for analysis while the other 13,883 children in the ALSPAC cohort do not.

DNA samples were available at three timepoints for the children: birth (cord blood), age 7 (peripheral blood), and age 15-17 (peripheral blood) (Relton et al., 2015). A full description of the methods used to create the epigenetic data and quality control procedures are found in Appendix B. In short, DNA methylation levels were assessed at roughly 485,000 CpG sites and were reported as a beta value ($\beta$). Beta values can range from 0 to 1 and represent the ratio of methylated probes to unmethylated probes at a specific CpG. This research utilized the epigenetic data—$\beta$ values—obtained from the DNA samples extracted at age 7 ($N = 925$). See Figure 2 for a summary of participant selection.
Figure 2

Flowchart of participant selection for study

14,901 children in ALSPAC

1,018 children in ARIES

925 children in ARIES with DNAm data at age 7

894 children in final ARIES analytic sample

13,883 children not included in ARIES

93 children in ARIES without DNAm data at age 7

31 children removed from ARIES because of extreme outlier in any variable
Measures

**Independent Variable Created: Neighborhood Stress**

The state of the mothers/child’s neighborhood was assessed via a four-point Likert questionnaire (1=serious problem, 4=no opinion) when the study child was 21, 31, 61, and 85 months old. The 11 items assessing neighborhood stress included questions regarding various facets like the state of the home (e.g., badly fitting doors) and worries regarding attacks and burglaries (Avon Longitudinal Study of Parents and Children Carer Questionnaire, 2022). The item reliabilities were good, see Table 3 for descriptive statistics and reliability coefficients for the timepoints and see Appendix A for the wording regarding the specific items.

A composite score was calculated at each timepoint by averaging the 11 neighborhood stress items. A participant response of “no opinion” on an item was not included as part of the composite. Each score was then reverse coded so higher numbers represent a greater amount of neighborhood stress. A cumulative neighborhood stress score then was calculated for each participant by calculating the mean of the composite neighborhood stress scores at each time point (i.e., 21, 31, 61, and 85 months). The resulting neighborhood stress score represents the average amount of neighborhood stress experienced over the previous seven years. The cumulative neighborhood stress score was subsequently standardized with a mean of 0 and standard deviation of 1.
Table 3

Descriptive statistics for neighborhood stress variable

<table>
<thead>
<tr>
<th>Timepoint (months)</th>
<th>N</th>
<th>Reliability coefficient</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>830</td>
<td>.784</td>
<td>.3546</td>
<td>.2964</td>
<td>0-2.0</td>
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<tr>
<td>31</td>
<td>816</td>
<td>.799</td>
<td>.3523</td>
<td>.3042</td>
<td>0-2.0</td>
</tr>
<tr>
<td>61</td>
<td>806</td>
<td>.770</td>
<td>.2960</td>
<td>.2789</td>
<td>0-1.92</td>
</tr>
<tr>
<td>85</td>
<td>816</td>
<td>.748</td>
<td>.2587</td>
<td>.2498</td>
<td>0-1.67</td>
</tr>
</tbody>
</table>

Independent Variable Created: Financial Difficulties

The state of the mother’s ability to afford basic needs for the child was assessed at three child time points: 8 months, 33 months, and 85 months. A four-point Likert scale, (ranging from “1 = very difficult” to “4 = not difficult”) was used to gauge the difficulty that the mother had affording food, clothes, heating, and rent or mortgage for the child. In addition, ability to afford educational courses, medical or dental, childcare, and other things were items that were added to the questionnaire administered at 85 months (Avon Longitudinal Study of Parents and Children Carer Questionnaire, 2022). See Appendix A for the wording of the specific items.

The item reliabilities were good at the 8 month, 33 month, and 85 month time points, see Table 4 for alpha coefficients and other descriptive metrics for the financial difficulties variable. The mean was calculated at each time point to compute a financial difficulty score. Each score was then reverse coded so higher numbers represent a greater amount of financial difficulty.

Financial difficulties was calculated by finding the mean of the financial difficulty scores from
the three time points. The resulting score represents the difficulty the mother had affording basic needs of the child during the first seven years of the child’s life. This mean financial difficulty score was subsequently standardized with a mean of 0 and standard deviation of 1.

Table 4

Descriptive statistics for financial difficulties variable

<table>
<thead>
<tr>
<th>Time point</th>
<th>N</th>
<th>Reliability coefficient</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
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<tr>
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<td>.5462</td>
<td>.6760</td>
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<tr>
<td>33 months</td>
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<td>.840</td>
<td>.4862</td>
<td>.6367</td>
<td>0-2.8</td>
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<tr>
<td>85 months</td>
<td>790</td>
<td>.860</td>
<td>.31.82</td>
<td>.4734</td>
<td>0-3.0</td>
</tr>
</tbody>
</table>

Validity of Neighborhood Stress and Financial Difficulty Independent Variables

The financial difficulties and neighborhood stress variables demonstrate adequate construct validity for several reasons; they are discussed next.

Face validity. The indicators of poverty included in this research possess face validity; that is, neighborhood stress and financial difficulties probably capture and accurately assess the construct of poverty. Particularly of note is that the poverty indicators all indicate a degree of deprivation or want which is consistent with Boltvinik’s (1999) definition of poverty.

Content validity. Lok Dessallien (1999) reported that there are several groups of poverty indicators: income, basic needs, human capability (i.e., longevity), and “other” (e.g., land, access to information, and access to social and physical infrastructure.). The poverty variables used in this research demonstrate good content validity because the financial difficulties and neighborhood stress variables “cover” these subdomains of poverty. For instance, financial
difficulties allude to not only struggling to afford basic needs like food and clothes, but also the underlying cause of the financial difficulties themselves, a lack of money. Or consider that the neighborhood stress variable assesses the poverty construct through Lok Dessallien’s “other” poverty group; many of the items on the measure assess the quality and safety of the neighborhood. To sum, the indicators of poverty utilized in this research match the definitions developed by experts in the field (i.e., Lok Dessallien) and therefore demonstrate content validity.

Convergent validity. The indicators of poverty utilized in this research demonstrate convergent validity because they are correlated with other constructs that theory infers they should be. For example, theory suggests that people living in poverty may be more prone to depression and anxiety (Ridley et al., 2020). Within the ARIES subsample, financial difficulties is correlated with the Edinburgh Postnatal Depression Score (Cox et al., 1987) \( r = .352, \ p < .001 \), Crown Crisp Depression subscale \( r = .313, \ p < .001 \), and the Crown Crisp Anxiety subscale (Crown & Crisp, 1979) \( r = .342, \ p < .001 \). Similarly, neighborhood stress is correlated with the Edinburgh Postnatal Depression Score \( r = .290, \ p < .001 \), Crown Crisp Depression subscale \( r = .265, \ p < .001 \), and the Crown Crisp Anxiety subscale \( r = .251, \ p < .001 \).

To summarize, the financial difficulties and neighborhood stress independent variables are valid because they accurately assess the poverty construct. In other words, the independent variables utilized in this research adequately assess and capture the poverty construct within the ARIES data set.

Final Independent Variable Created: Poverty

The standardized financial difficulties and standardized neighborhood stress measures were averaged to create the poverty variable. The higher the poverty variable value for each
participant, the greater the financial difficulties and neighborhood stress, that is, poverty, experienced by each participant in the first 7 years of their life.

**Justification For Evaluating ACE Variables as an Accumulated Exposure**

Examining adversity as an independent variable can occur within different methodological contexts. The influence of adversity on outcomes has traditionally been assessed either as an adversity exposure accumulation measure (i.e., adversity exposure amount over time as an accumulated quantity or average) or as an exposure to adversity during sensitive periods measure (i.e., a binary measure indicating a yes/no to exposure during different sensitive windows). A more recent approach to examining adversity in childhood is to model the change in adversity—that is, as a trajectory. Although sensitive windows may exist in childhood for adversity to influence DNAm patterns (e.g., Dunn et al., 2019), and although the amount of adversity may change throughout childhood (e.g., Tracey et al., 2019), this research examined the ACE independent variable as an accumulated measure.

An ACE accumulated measure is appropriate for this study for several reasons. First, the sensitive window hypothesis has been previously evaluated within the ALSPAC cohort: Marini et al., (2020) reported null associations between various types of adversity exposures during sensitive windows in childhood and epigenetic age. Specifically, financial difficulties and neighborhood disadvantage during sensitive windows were not associated with epigenetic age as determined by the Horvath Clock (Marini et al., 2020). Further, most research examining the influence of ACEs on epigenetic age examines adversity types as accumulated measures (e.g., Tang et al., 2020 within the ALSPAC cohort)—Marini et al., (2020) is, in fact to date, the lone exception.
Second, examining the ACE variable included in this research as childhood adversity trajectory semantically changes the research question. This research aimed to investigate whether adversity during childhood—as measured by indicators of poverty— influenced LoC and is mediated by epigenetic age. If investigating childhood adversity trajectories, the hypothesis morphs to become a question regarding how changes to childhood adversity—or lack thereof—influences LoC and is mediated by epigenetic age. The semantic difference lies within the “if poverty was experienced” versus “does a change in poverty” distinction.

Moreover, a recent investigation using the ALSPAC cohort evaluating childhood adversity trajectories and risk of depression noted that three of the five identified trajectory groups—those involving moderate to high adversity—shared similar associations with depression risk and severity (Tracey et al., 2019). The inference to draw from this observation is that the trajectory-based modeling technique provided little additional insight into the main conclusion drawn from the study—that childhood adversity is associated with depression. Similar inferences can be drawn regarding the efficacy of adversity trajectories in yielding insights and conclusions that surpass other means of modeling the same relationship (Suglia et al., 2012) (e.g., accumulation or mean based measures). To sum, trajectories may be unnecessary to evaluate the relationship between ACEs and LoC.

**Dependent Variable Created: Locus of Control**

A modified 12 item version of the Children’s Nowicki-Strickland Internal-External scale (CNSIE) was administered to the children when they were about 89 months old (Avon Longitudinal Study of Parents and Children Clinical measures, 2022). To develop the modified 12 item version of the CNSIE, the full version of the CNSIE, (i.e., 40 items), was administered to a sample of children and the 12 items with the highest item-total correlation were used to create
the modified version (Nowicki & Strickland, 1973). The CNSIE has been found to have good construct validity and test-retest reliabilities within this age group (Nowicki, 1976; Nowicki & Duke, 1974).

Administration of the CNSIE occurred at a clinic by a trained examiner. The instructions were described and the fact that there are no correct answers emphasized. The child responded to statements read by the examiner in a “yes/no” fashion. See Appendix A for the wording of each statement. A response of “yes” was coded as “1” and a response of “no” was coded as “0.” The score of each item was summed to create a locus of control aggregate score. Higher numbers on the locus of control aggregate score indicate a more external LoC.

The mean LoC was 5.91 (SD = 1.98) and ranged from 0 to 11. Split-half Spearman-Brown coefficients indicate that item reliabilities were low ($r = .47$). Removing items did not improve the reliability coefficient. Although internal consistencies were low, there are several reasons that it was appropriate to keep locus of control for the analyses. First, Nowicki and Strickland (1973) reported that internal consistency of the full length CNSIE was moderate—$r = .63$—but noted that the internal consistency was satisfactory because the items are not arranged by difficulty and the items are additive. Second, Nowicki and Strickland (1973) suggested that the split-half method underestimates the true internal consistency of a measure. Last, several papers have been authored by the developer of the CNSIE that use the ALSPAC cohort LoC data. Nowicki et al. (2018a) and Nowicki et al. (2018b), for instance, have investigated the LoC construct within the ALSPAC cohort and while the internal consistencies were not reported, the fact that the CNSIE developer used the ALSPAC LoC data is evidence that the LoC construct may be considered reliable. To sum, while internal consistency is low in this study, using the rationale proposed by the developers of the CNSIE—that lower reliability coefficients are
satisfactory because the items are not arranged by difficulty and are additive—and considering the usage of the ALSPAC LoC construct by the developer of the CNSIE, the LoC construct was used with confidence in this research.

**Mediator Variable Created: Epigenetic Age at 7 years**

Epigenetic age at age 7 was assessed using the Horvath Clock, a widely used calculator of DNA methylation age calculator (Field et al., 2018; Horvath & Raj, 2018) developed by S. Horvath (Horvath, 2013; https://dnamage.genetics.ucla.edu/).

The Horvath DNA methylation age calculator was developed using a machine learning method, in this case an elastic net regression, that produced a linear regression model when a transformed version of chronological age was regressed on a training set of DNA methylation data. The linear regression model includes the most informative CpGs—the “clock CpGs” (Horvath, 2013)—and their relative influence. In other words, the machine learning analysis produced a linear regression model with two things (a) a set of 353 CpGs that are each correlated with age, and (b) a corresponding regression weight coefficient for each CpG (Horvath, 2013). The regression weight coefficient (i.e., \( b_1, b_2 \cdots b_{353} \)) represents the amount of DNA methylation that changes as a function of chronological age (Field et al., 2018). The calculator computes DNA methylation age entering the beta values of 353 specific CpGs into the linear regression model. The predicted DNA methylation age is the sum of the products of the 353 clock CpG regression weight coefficients with their respective DNA methylation beta values:

\[
\text{DNAmAge} = \text{inverse.F}(b_0 + b_1 \text{CpG}_1 + \cdots + b_{353} \text{CpG}_{353})
\]

(Horvath, 2013). The Horvath clock has been shown to be a valid predictor of chronological age—the correlation between the predicted DNA methylation age based on the Horvath Clock and chronological age is over .90 (Horvath, 2013).
The mean DNA methylation age for the sample used in this research was 8.87 \((N = 925, SD = 2.87)\) and ranged from 2.80 to 29.33 years old.

**Analysis Plan: Does Epigenetic Age Mediate the Relationship Between ACEs and LoC?**

Analysis proceeded in four main steps. First, pre-analysis linear regression assumptions were evaluated and potential confounding variables were identified. Second, the nature and proportion of missing data was evaluated. Missing values were subsequently imputed. Third, statistical analysis utilizing the PROCESS macro (Hayes, 2017) was utilized to evaluate the indirect effect of the mediation. Fourth, a sensitivity analysis to evaluate gender and individual indicators of poverty was conducted. The procedures involved in each is described in detail next.

**Potential Confounding Variables**

The following variables were included as covariates:

1. *Prenatal exposure to tobacco smoke (smoking).* Prenatal exposure to tobacco smoke was reported to have an association with changes to DNAm at age 7 within the ALSPAC cohort (Richmond et al., 2015). Prenatal smoking was assessed when expectant mothers were 18 weeks pregnant. Smokers were coded as 1, nonsmokers as 0.

2. *Birthweight.* Birthweight was reported to have an association with epigenetic age at age 7 within the ALSPAC cohort (Simpkin et al., 2016). Preferred birthweight was assessed at childbirth for each participant.

3. *Ethnicity:* Ethnicity is a known correlate with epigenetic age (e.g., Horvath et al. 2016). Caucasian was coded as 0, all others were coded as 1.
4. **Gender**: Gender is a known correlate of epigenetic age (Horvath et al., 2016) and LoC (Ross & Mirowsky, 2002; Culpin et al., 2015). Males were coded as 1, females as 2. Gender was derived from the Horvath clock output at age 7.

**Pre-analysis Assumptions Testing**

Prior to analysis, the following steps were taken to assess the assumptions necessary to conduct the regression analyses needed to evaluate a mediation:

1. **Outliers**: Outliers were evaluated in the continuous variables by examining a z-score range of -3.29 - 3.29 (Tabachnick et al., 2019).

2. **Multicollinearity**: Multicollinearity between the predictors was evaluated using bivariate correlations and standardized residual values.

3. **Distribution and normality**: The skewness and kurtosis values of the distributions were evaluated using the skewness and kurtosis z-scores. The Kolmogorov-Smirnov and Shapiro-Wilk tests were also used to assess normality.

4. **Linearity**: Linearity was evaluated using the P-P plot.

5. **Homogeneity of variances**: Homoscedasticity was evaluated by examining the scatter plot of the standardized residuals of the predicted and observed values.

**Missing Cases and Imputation.** To examine selection bias, both the proportion of missing data and the nature of missing data was evaluated. Subsequently, 100 iterations of multiple imputation were used to replace the missing values. Multiple imputation has been shown to reduce bias, as compared to other imputation methods, in outcome metrics (Schlomer et al., 2010). The imputed data was aggregated into a final analytic data set.
**Statistical Analysis**

IBM SPSS v.25 and the macro PROCESS (Hayes, 2017) was used to conduct a series of regression analyses to evaluate the hypothesis that *epigenetic age* mediates the relationship between *poverty* and *locus of control*.

**Mediation.** The indirect effect (*ab*) was calculated and evaluated using the *PROCESS* macro for SPSS developed by Andrew Hayes (2017). Specifically, bootstrapped (N=5,000) confidence intervals for the indirect effect were calculated. Evaluating the indirect effect, in lieu of the traditional means of assessing a mediation—that is, the causal steps outlined by Baron and Kenny (1986)—is justified because while the Baron and Kenny (1986) is widely used, it has several limitations. First, as compared to assessing the indirect effect, the Baron and Kenny causal steps is not only prone to both Type I and Type II errors, but also suffers from low power (Preacher & Hayes, 2004). Moreover, it has been argued that assessing *ab* is a more direct approach to assessing the mediation than a series of regression analyses (Preacher & Hayes, 2004).

**Sensitivity Analysis**

The final analytic data set was split by the variable *gender*. The mediation was subsequently evaluated in each gender stratified subset. Further, the individual poverty predictors—*financial difficulties* and *neighborhood stress*—were evaluated in the mediation model independently.

**Power Analysis**

A *post hoc* power analysis was conducted to ensure that the sample size in the current study was sufficient. A traditional *a priori* power analysis was unnecessary for planning purposes because an *a priori* power analysis is useful when the sample size of a study is in
question. In other words, an *a priori* power analysis is used for sample size planning purposes. As an SDA, the sample size for this study was not in question and thus conducting an *a priori* power analysis was irrelevant because the sample size was already determined. With that noted, however, an *a priori* power analysis was conducted to determine what effect sizes the model was able to detect (van den Akker et al., 2020). Using the simulation values reported by Fritz and MacKinnon (2007), a sample of $N = 894$, a power of .8 can detect small effect size for the $a$ coefficient and the $b$ coefficient. The minimum sample size to detect small effect sizes for both coefficients is $N = 558$ (Fritz and MacKinnon, 2007),
CHAPTER FOUR
RESULTS

This study evaluated the potential for epigenetic age to mediate the relationship between poverty and locus of control in $N = 894$ children. Evaluating the model occurred in three main steps. First, pre-analysis linear regression assumptions were evaluated. Second, the nature and proportion of missing data was evaluated. Missing values were subsequently imputed. Third, statistical analysis evaluated the indirect effect of the mediation. The results of each are discussed next.

Pre-analysis Linear Regression Assumptions Evaluation: Assumption Violations Detected

Prior to the evaluation of the mediation model, assumptions for regression analysis were evaluated.

Outlier Evaluation

Each continuous variable was evaluated for outlier values. Outliers were evaluated in the financial difficulties and neighborhood stress variables prior to creating the poverty composite variable. Three cases exceeded 3.29 standard deviations in the financial difficulties variable and 13 cases exceeded the maximum value in the neighborhood stress variable. The outlier cases were deleted from the data set. The locus of control variable was evaluated for outliers by examining z-scores; no outliers were detected. Epigenetic age was evaluated for outlier values. Twelve cases exceeded the acceptable range (i.e., -3.29-3.29). The outlier cases were deleted from the data set. Birthweight was evaluated for outlier values using z-scores. Three cases exceeded the acceptable range (i.e., -3.29-3.29) and were deleted from the data set. A total of 31 extreme outlier cases were removed from the final analytic sample resulting in $N = 894$. 

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Assumption of Normality

The linear regression assumption that variables are normal was evaluated using skewness z-scores, kurtosis z-scores, the Kolmogorov-Smirnov test, the Shapiro-Wilk test, and histograms.

The poverty variable was not normal. Both the skewness and kurtosis z-scores exceeded the maximum z-score value 1.96. Further, both the Kolmogorov-Smirnov test and the Shapiro-Wilk were found to be significant, indicating nonnormality. The appearance of the histogram confirmed that the poverty variable was not normal and positively skewed. To address the violation of normality, a reciprocal transformation was performed and did not remedy the violation.

The locus of control dependent variable was generally normal. The skewness z-score did not exceed the acceptable maximum value. It was, however, slightly kurtotic with a kurtosis z-score that marginally exceeded the maximum value of 1.96. Both the Kolmogorov-Smirnov test and the Shapiro-Wilk were found to be significant.

The mediator variable, epigenetic age, was not normal. Both the skewness and kurtosis z-scores exceeded the maximum value 1.96. Further, both the Kolmogorov-Smirnov test and the Shapiro-Wilk test were found to be significant, indicating nonnormality. The appearance of the histogram confirmed that the variable is skewed. To address the violation of normality, square root and Log10 transformations were performed. The square root transformation did not remedy the assumption violation. The Log10 transformation, however, slightly improved the distribution—the results of the Kolmogorov-Smirnov test became not significant ($p = .065$). Thus, the Log10 poverty was used in the mediation analysis.
The covariate *birthweight* was normally distributed. The appearance of the histogram was normal and the skewness and kurtosis \( z \)-score values did not surpass the maximum acceptable value. Further, the Kolmogorov-Smirnov test and the Shapiro-Wilk test were not significant, indicating normality.

**Multicollinearity Assumption**

There existed no multicollinearity between the independent variable *poverty* and the mediator variable *epigenetic age*. First, the bivariate correlation was not significant. Second, the standardized residuals fell within the acceptable range. There exists no multicollinearity between the covariate variables. See Table 5 for Pearson correlation coefficients.

**Table 5**

*Correlation matrix*

<table>
<thead>
<tr>
<th></th>
<th>Poverty</th>
<th>Epigenetic Age</th>
<th>Locus of Control</th>
<th>Birthweight</th>
<th>Gender</th>
<th>Smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epigenetic Age</td>
<td>.044</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locus of Control</td>
<td>.021</td>
<td>-.015</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight</td>
<td>.003</td>
<td>.074*</td>
<td>-.008</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>.040</td>
<td>-.070*</td>
<td>.053</td>
<td>-.138**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>.193**</td>
<td>.011</td>
<td>.041</td>
<td>-.081*</td>
<td>-.020</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td>-.073*</td>
<td>-.010</td>
<td>.077*</td>
<td>.083*</td>
<td>-.047</td>
<td>-.054</td>
</tr>
</tbody>
</table>

*Correlation is significant at \( p=.05 \) level

**Assumption of Linearity**

A Normal P-P plot was examined to evaluate the linearity of the relationship between the predictors and dependent variable. The Normal P-P plot indicated that the mediator variable,
epigenetic age, did not have a linear relationship with the dependent variable, locus of control. Examination of the scatter plot further indicated that the relationship was not only not linear, but also did not appear to be a form of nonlinear relationship. That is, the relationship between the mediator variable epigenetic age and the dependent variable locus of control appeared to be random.

A Normal P-P was examined to evaluate the linearity of the relationship between the independent variable poverty and the dependent variable locus of control. The P-P plots indicated that the relationship was not linear; the scatterplot of the two variables confirmed this observation and demonstrated a nonlinear relationship.

Assumption of Homogeneity of Variance

Homoscedasticity was evaluated by examining the scatter plot of the standardized residuals after entering the independent variable poverty and the mediator variable epigenetic age into a regression model predicting the dependent variable locus of control. The scatter plot generally appeared rectangular which indicates that the residuals are random.

Addressing Violations of Assumptions: Bootstrapping

The variables used in the mediation model were not normal and the relationship between the mediator, epigenetic age, and the dependent variable, locus of control, was not linear. To address the violations of assumptions, as noted, various transformations were performed and were limited in their success. Since the data were not normally distributed and other assumptions of regression were violated, a bootstrapping technique was used that is robust to nonnormal data (Erceg-Hurn & Mirosevich, 2008). Inherent in the PROCESS macro is a bootstrapping step (Hayes, 2017). Bootstrapping is a resampling method known to be unaffected
by data that violates assumptions of normality and linearity and is commonly used to evaluate
data drawn from nonparametric populations (Field, 2013).

**Missing Data: Small Proportion of Data Missing Completely at Random**

The nature and proportion of missing data was evaluated. Since this research was
longitudinal and incorporated several composite measures (i.e., various measures developed
using numerous items at various timepoints), missing data was expected (Ibrahim &
Molenberghs, 2009). In other words, the poverty variable used in this research was a composite
of financial difficulties and neighborhood stress composite measures. The financial difficulties
and neighborhood stress composite measures are themselves composite measures of numerous
items assessed at various timepoints. An incomplete item, at any point, constituted a missing
value and thus contributed to the overall “missingness” of the data. Others have noted high levels
of missing data—particularly regarding adversity measures—with in the ALSPAC cohort (e.g.,
Houtenpen et al., 2018).

**Proportion and Nature of Missing Data**

Of the ARIES final analytic sample ($N = 894$) (i.e., after removing extreme outliers),
22.48% of cases ($N = 201$) were missing at least one of the seven variable values. This amounted
to small, albeit significant proportion: Of the total number of values possible in the data set,
3.85% were missing ($N = 241$). A Little’s MCAR Test revealed that within the continuous
variables (i.e., poverty, locus of control, epigenetic age, and birthweight), the missing values
were missing completely at random, $\chi^2(16) = 14.619, p = 0.553$. Within the categorical variables,
there was no difference in missing data between males and females $\chi^2 (1) = 1.313, p = 0.252$ and
whether the child’s mother smoked during pregnancy $\chi^2 (1) = 0.412, p = 0.521$. 
The variable with the greatest amount missing data was *locus of control*: 18.9% ($N = 169$). See Table 6 for a summary of the missing values for the other variables. The mean *locus of control* score for people with missing data was 6.31 ($SD = 1.75$) and the mean *locus of control* score for people without missing data was 5.88 ($SD = 2.00$). This difference was not significant $t(723) = -1.208, p > .05$. The mean *epigenetic age* for people with missing data was 8.74 ($SD = 2.28$) and the mean *epigenetic age* for people without missing data was 8.69 ($SD = 2.46$). This difference was not significant $t(892) = -0.284, p > .05$. The mean *poverty* score for people with missing data was 0.016 ($SD = 0.84$) and the mean *poverty* score for people without missing data was 0.009 ($SD = 0.80$). This difference was not significant $t(874) = -0.105, p > .05$. The mean *birthweight* for people with missing data was 3463.60 grams ($SD = 464.06$ grams) and the mean *birthweight* for people without missing data was 3501.70 grams ($SD = 469.84$ grams). This difference was not significant $t(874) = 0.978, p > .05$.

To sum, data was missing completely at random and independent of *gender*, *birthweight*, *locus of control*, *epigenetic age*, and *poverty* score.

**Table 6**

*Number of cases with missing values per variable*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number Valid</th>
<th>Number Missing</th>
<th>Percentage Missing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poverty</td>
<td>876</td>
<td>18</td>
<td>12%</td>
</tr>
<tr>
<td>Epigenetic Age</td>
<td>894</td>
<td>&lt; 5</td>
<td>&lt; 5%</td>
</tr>
<tr>
<td>Locus of control</td>
<td>725</td>
<td>169</td>
<td>18.9%</td>
</tr>
<tr>
<td>Birthweight</td>
<td>876</td>
<td>18</td>
<td>2.0%</td>
</tr>
<tr>
<td>Gender</td>
<td>894</td>
<td>&lt; 5</td>
<td>&lt; 5%</td>
</tr>
<tr>
<td>Smoking</td>
<td>880</td>
<td>14</td>
<td>1.6%</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>872</td>
<td>22</td>
<td>2.5%</td>
</tr>
</tbody>
</table>
Mediation Analysis: No Evidence For the Indirect Effect of Epigenetic Age

The macro PROCESS (Hayes, 2017) in SPSS was used to evaluate the mediation model. Prior the model evaluation, participant demographics were summarized.

Descriptive Statistics and Sample Characteristics

See Table 7 for a summary of participant characteristics. The final analytic sample—$N = 894$ children— contained $N = 441$ males (49.3%) and $N = 453$ females (50.7%). The sample was mostly white (98.3%). Of the final analytic sample, 13.5% ($N = 121$) experienced extreme poverty, defined as scoring at least one standard deviation above the mean. A total of $N = 488$ (55.9%) had an external LoC, that is, a LoC score that fell between the range of 6-11. Overall, 22.8% ($N = 204$) had a negative epigenetic age acceleration (i.e., their predicted chronological age was less than 7) and 73.8% ($N = 690$) had a positive epigenetic age (i.e., their predicted chronological age was greater than 7). Lastly, $N = 111$ (12.4%) of the children had mothers who smoked during pregnancy.

See Table 5 for a summary of correlation coefficients. Within the ARIES sample, poverty was positively associated with smoking $r = .193, p < .001$ and associated with being a minority $r = -.073, p = .031$. Epigenetic age was positively correlated with birthweight $r = .074, p = .029$ and negatively correlated with the female gender $r = -.07, p = .037$. Locus of control was positively correlated with ethnicity $r = .077, p = .039$; Caucasian participants tended to have a higher LoC. Birthweight was associated with female babies $r = -.138, p < .001$, prenatal smoking $r = -.081, p = .016$, and being a minority $r = .083, p = .015$. 

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Table 7

Descriptive statistics for participants in the final analytic sample N=894

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre missing data imputation</th>
<th>Post missing data imputation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean/Count</td>
<td>SD</td>
</tr>
<tr>
<td>Poverty (z-scores)</td>
<td>.0086</td>
<td>.805</td>
</tr>
<tr>
<td>Epigenetic Age (LOG10)</td>
<td>.923</td>
<td>.125</td>
</tr>
<tr>
<td>LoC</td>
<td>5.88</td>
<td>2.00</td>
</tr>
<tr>
<td>Birthweight</td>
<td>3501.7</td>
<td>469.84</td>
</tr>
<tr>
<td>Sex</td>
<td>M: 441</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F: 453</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>No: 769</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes: 111</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td>White: 870</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other: &lt; 5</td>
<td></td>
</tr>
</tbody>
</table>

**PROCESS Mediation Analysis**

See Figure 3 for a summary of coefficients in the mediation model and Table 8 for a summary of the direct and indirect model parameters from the mediation analyses. The adjusted model contained the predictor, poverty, the dependent variable, locus of control, the mediator, epigenetic age, and the covariates (i.e., birthweight, gender, smoking, ethnicity). There was no evidence that epigenetic age mediated the relationship between poverty and locus of control, indirect effect $\beta = -0.002$, 95% bootstrap CI [-0.012, 0.007]. In other words, the indirect effect of poverty on locus of control through epigenetic age was not significant: there is no difference between the population indirect effect of epigenetic age on the relationship between poverty and
locus of control and 0. Moreover, there was no evidence of an influence of poverty on locus of control (β = 0.050, t = .649, p = 0.517, CI [-0.101, 0.201]), no evidence that poverty had an influence on epigenetic age (β = 0.007, t = 1.28, p = 0.210, CI [-0.004, 0.017]) and no evidence that epigenetic age influenced locus of control (β = 0.233, t = .467, p = 0.640, CI [-1.210, 0.745]).

When the covariates were removed from the model, the indirect effect of the unadjusted model remained 0, indirect effect β = -0.013, 95% bootstrap CI [-0.013, 0.006].

**Figure 3**

*Parameter estimates for mediation model*

![Diagram showing mediation model with parameter estimates for epigenetic age and locus of control.]

**Sensitivity Analysis.** Males and females were analyzed separately because there are known associations between gender and epigenetic age (Simpkin et al., 2016) and locus of control (Culpin et al., 2015). Within males, there was no evidence that epigenetic age mediated the relationship between poverty and locus of control, indirect effect β = -0.0001, 95% bootstrap CI [-0.013, 0.012]. Within females, there was no evidence that epigenetic age mediated the
relationship between poverty and locus of control, indirect effect $\beta = -0.006$, 95% bootstrap CI [-0.029-0.012].

There were no group differences in LoC orientation or poverty experienced. Males, however, had a slightly higher epigenetic age than females and this difference in epigenetic age was marginally significant $t(892) = 2.089, p = .037$. Further, poverty, epigenetic age, and locus of control were not correlated within males. Within females, however, while other correlations were not significant, one association—that between poverty and epigenetic age—was borderline $r = .089, p = .058, 95\% \text{ CI } [-.006 - .184]$. Females that experienced higher levels of poverty tended to have higher epigenetic ages.

While this research created a cumulative ACE measure—poverty—it is common to evaluate individual ACE indicators (e.g., Culpin et al., 2015; Jovanovic et al., 2017). The variables financial difficulties and neighborhood stress were also evaluated individually. In the adjusted model, there was no evidence that epigenetic age mediated the relationship between neighborhood stress and locus of control, indirect effect $\beta = -.0006$, 95% bootstrap CI [-.007, .005]. When the covariates were removed, the indirect effect remained 0. Further, in the adjusted model, there was no evidence that epigenetic age mediated the relationship between financial difficulties and locus of control, indirect effect $\beta = -.0011$, 95% bootstrap CI [-.009, .006]. When the covariates were removed, the indirect effect of financial difficulties on locus of control through epigenetic age remained 0.

Post-hoc Power Analysis. To ensure that the null findings were not a result of insufficient sample size, Fritz and Mackinnon (2007) was consulted. Using the observed sample sizes and

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1 A power analysis using G*Power is not possible for a mediation model using the PROCESS macro for SPSS because the distribution of the indirect effect $ab$ is not normal. Thus, the simulation table—Table 3—from Fritz and Mackinnon (2007) was consulted. The indirect effect, $ab$, is not normal because the product of two variables with normal distributions, in this case $ab$, is not normally distributed. For more information regarding the nonnormal distribution of the indirect $ab$, see Fritz and MacKinnon (2007), MacKinnon et al., (2004)
the percentile bootstrap simulation output (i.e., Fritz & MacKinnon, 2007), it was determined that the current study had an appropriate sample size and the null findings are not the result of insufficient power.

Table 8

Estimates N=894 of the direct and indirect effects of poverty on locus of control mediated through epigenetic age

<table>
<thead>
<tr>
<th>Model Estimates</th>
<th>β</th>
<th>SE</th>
<th>p</th>
<th>95% CI(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unadjusted</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Effect</td>
<td>0.048</td>
<td>0.075</td>
<td>0.525</td>
<td>-0.099-0.195</td>
</tr>
<tr>
<td>poverty on locus of control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indirect Effect</td>
<td>-0.021</td>
<td>0.005</td>
<td></td>
<td>-0.013-0.006</td>
</tr>
<tr>
<td>poverty on locus of control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>through epigenetic age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Adjusted(^b)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Effect</td>
<td>0.050</td>
<td>0.077</td>
<td>0.517</td>
<td>-0.101-0.201</td>
</tr>
<tr>
<td>poverty on locus of control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indirect Effect</td>
<td>-0.002</td>
<td>0.004</td>
<td></td>
<td>-0.012-0.007</td>
</tr>
<tr>
<td>poverty on locus of control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>through epigenetic age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) 5000 Bootstrap samples

\(^b\) adjusted for gender, ethnicity, birthweight, and maternal smoking
CHAPTER FIVE

DISCUSSION

Childhood adversity influences different facets of cognition, like locus of control. How adverse experiences, such as poverty, get under the skin to influence locus of control, however, is unknown. In this study, epigenetic age was evaluated as a potential mechanism linking poverty to locus of control. Specifically, this study evaluated the potential for epigenetic age to mediate the relationship between poverty and locus of control in $N=894$ children at age 7.

Previous research supports the proposition that epigenetic age could be a mechanism by which poverty influences locus of control. Specifically, there is evidence that poverty influences locus of control (Mittal & Griskevicius, 2014; Wickline et al., 2011), that poverty influences epigenetic age (Chen et al., 2016; Peng 2019), and that epigenetic age may influence facets of cognition (Tollenaar et al., 2021). Moreover, a recent report indicated that epigenetic age mediated the relationship between childhood adversity and a pathology of cognition, specifically depressive symptoms (Sumner et al., 2019). Collectively, these bodies of research made the hypothesis that the relationship between poverty and locus of control is mediated by epigenetic age plausible.

To evaluate the mediational model, a secondary data analysis was performed using the ARIES cohort within the ALSPAC data set. Adversity measures were collected at various timepoints between ages 1 and 7 during childhood and locus of control and epigenetic age data were collected at approximately age of 8 and 7, respectively. The SPSS macro PROCESS (Hayes, 2017) was used to evaluate the indirect effect of poverty on locus of control through epigenetic age. It was hypothesized that the bootstrapped confidence intervals for the indirect
effect would not include 0 and thus that the relationship between poverty and locus of control would be, at least, in part due to the influence of epigenetic age.

Results showed that epigenetic age did not mediate the relationship between poverty and locus of control. Moreover, gender stratified analyses yielded the same results, epigenetic age did not mediate the relationship between poverty and locus of control in males or females. All associations remained null even when the components of the poverty variable—financial difficulties and neighborhood stress—were evaluated independently. This suggests that, within the ARIES cohort, neither ACEs, operationalized as poverty, nor epigenetic age, using the Horvath Clock, influences LoC. In total, regardless of how the variables were parsed and evaluated, there was not only little evidence of epigenetic age mediating the relationship between poverty and locus of control, but there was also very little evidence of any relationship between any of the variables. This is in stark contrast to the literature, discussed throughout the Introduction and Literature Review that suggests the opposite.

**Major Findings**

Given the null findings, there are at least six major inferences that can be drawn from this study. These are (1) the severity of the adversity may matter, (2) the influence of poverty may not be absent, it may just take time to develop, (3) the type of poverty indicator may matter, (4) the type of epigenetic age calculator may matter, (5) epigenetic age may not be related to locus of control, and (6) males have a higher epigenetic age and female participants may be more sensitive to adversity. Each inference is discussed below within its own section. This chapter then concludes with a section describing the strengths and limitations of the current study.

**The Severity of The Adversity May Matter**

Once inference from the null findings may be that poverty is not a severe enough
adversity to influence epigenetic age or LoC. There are several reports whose findings support this inference.

In parallel with the current study, Sumner et al., (2019) reported null associations between deprivation—operationalized as indicators of poverty like food insecurity and neglect—and epigenetic age. Sumner and colleagues (2019), however, also reported associations between threat exposure and epigenetic age. Threat exposure was operationalized as physical abuse, sexual abuse, emotional abuse, domestic violence, and other forms of interpersonal violence. In other words, within the cohort that Sumner and colleagues (2019) evaluated, abuse was related to epigenetic age, but indicators of poverty were not.

Moreover, while direct comparisons are difficult because Tang et al., (2020) evaluated conventional ACEs and not expanded ACEs like indicators of poverty, Tang and colleagues (2020) also reported that forms of abuse influenced epigenetic age. Specifically, Tang et al., (2020) reported associations in teenage females who experienced emotional and physical abuse and epigenetic age. Jovanovic et al., (2017) reported something similar, that directly experiencing violence was associated with epigenetic age.

Last, in a meta-analysis examining the independent associations of threat and deprivation on different measures of biological aging—like epigenetic age—Colich et al. (2020) report that epigenetic age was associated with childhood adversities characterized by threat—defined broadly and included adversities like emotional abuse, physical abuse, and observing domestic violence—but not SES or deprivation. From these studies, it may be inferred that severe adversity influences epigenetic age but less severe adversity does not.

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2 Colich et al. (2020) analyzed, along with epigenetic age, telomere length, another metric often used to assess biological aging. A total of 11 studies were analyzed, two of which specifically evaluated epigenetic age (Jovanovic et al., (2017); Sumner et al., (2019)).
The current study also highlights the role of the severity of adversity on LoC development. In other words, the severity of childhood adversity may also matter when evaluating the relationship between ACEs and LoC. For instance, also evaluating the ALSPAC cohort, Fisher et al. (2013) reported associations between childhood adversity and LoC at the same timepoint as the current study—age 8—but the adversity measures were different. That is, the current study operationalized poverty as an indicator of childhood adversity while Fisher et al. (2013) operationalized adversity using measures of domestic violence, child abuse, and extreme bullying. Other studies reporting associations between childhood adversity and childhood LoC operationalized childhood adversity in a similar manner as Fisher et al. (2013) (e.g., child maltreatment (Roazzi et al., 2016) and emotional neglect, psychological abuse, and physical abuse (Hovens et al., 2016)).

Taken together with these studies, the current research may suggest that severe types of adversity—perhaps those involving violence or abuse—are more influential on epigenetic age and LoC than poverty in early childhood. As it turns out, this conclusion is also supported by theory.

Levels of DNAm change as a function of severity, chronicity, and timing of adversity in genes involved with the stress response. (Cicchetti & Handley, 2017; Tyrka et al., 2015). Interestingly, many of the 353 CpGs that make up the Horvath Clock are mapped to genes involved with the stress response (Zannas et al., 2015). Therefore, perhaps, with greater adversity and stress, the DNAm levels of the CpGs comprising the Horvath Clock change which may result in a shift in epigenetic age. In other words, a stressful event results in a shift in epigenetic age because the genes involved in the stress response are the same genes involved in epigenetic age predication. Indeed, it has been proposed that circulating stress hormones (i.e.,
glucocorticoids) may mediate the relationship between stressful events and epigenetic age (Zannas et al., 2015). With reference to the current study and in context with this argument, the current findings suggest that poverty may not be a stressful enough stimulus to induce the changes just described.

**The Influence of Poverty May Take Time to Develop**

An alternative to the conclusion that poverty may not be stressful enough to influence epigenetic age is that poverty simply takes additional time to influence epigenetic age and LoC compared to more severe forms of adversity. In other words, poverty is not a potent enough stimulus to induce relatively acute change. To make an analogy: Perhaps severe adversities are like floods—changing a landscape quickly—while poverty is like a trickle—shaping a landscape over an extended time. In conjunction with the current study, there is evidence for this inference.

Consider that the current study evaluated the influence of indicators of poverty on LoC at age 8 and reported a null association while Culpin et al., (2015) reported a significant association between childhood socioeconomic adversity (i.e., poverty) and LoC at age 16 in the same cohort. An inference to draw from this difference is that the impact of poverty took more time to influence LoC; that is, the effect was negligible at age 8 but substantially increased to the point where it was significant and measurable in adolescence. Worth repeating is that the current study was adequately powered to measure such an effect at age 8 and thus it is plausible that the impact of poverty on LoC accumulated, or is delayed, as the children aged.

The same rationale may be applied to the influence of poverty on epigenetic age. While the current study found a null relationship between indicators of poverty and epigenetic age measured at age 7, Chen et al., (2016) reported a significant association between economic hardship and epigenetic age measured in adolescence. To reiterate, the influence of poverty, due
to its lack of severity, may take time to influence epigenetic age and could account for the null associations reported in the current study.

In sum, the null findings in the current study may be due to a lack of adversity severity. The current study, however, may also suggest that certain types of adversity are more influential not only on epigenetic age, but also on LoC. Last, it is possible that the influence of poverty on LoC and epigenetic age may develop with time. More research is needed to further examine these possibilities.

**The Type of Poverty Indicator May Matter**

In the current study, the primary analysis evaluated a cumulative ACE measure. Subsequent sensitivity analyses evaluated individual ACE measures. Not only was the mediation model evaluating the cumulative poverty measure found to be null, but so were the models evaluating the individual measures. In other words, when evaluated independently, both the financial difficulties and neighborhood stress mediation models were also found to be null. The forthcoming discussion will not only attempt to explain the null findings from the primary model, but also attempt to explain the null findings from the sensitivity analyses.

While the point has been made that poverty may not constitute a severe enough adversity to induce change, there are, however, studies that demonstrate that indicators of poverty do influence epigenetic age in children. A closer examination of these reports highlights another inference drawn from the current study: That the specific indicators of poverty may matter when investigating the relationship between poverty and epigenetic age. In other words, different indicators of poverty may influence epigenetic age while others do not.

For example, Jovanovic et al. (2017) reported that parental education and household income were predictive of epigenetic age. Similarly, Peng et al. (2019) also found that maternal
education was associated with epigenetic age in children. Last, Chen et al. (2016) reported that measures of household income were associated with epigenetic age. Each study included either a measure of parental education, a measure of income, or both. The current study did not include a measure of parental education or a measure of household income but instead justified and included measures of financial difficulties and neighborhood stress and the indicators of poverty.

To revisit a theme in the previous sections, perhaps the severity of poverty indicators like income and parental education are enough to influence epigenetic age and LoC while financial difficulties and neighborhood stress are not—at least not in early childhood.

Moreover, there is also evidence that poverty, operationalized by income and parental education, is associated with LoC (Ahlin, 2014; Mittal & Griskevicius, 2014; Pedron et al., 2021; Wickline et al., 2011). It may be argued that several studies also included measures similar to financial difficulties and neighborhood stress—like Wickline et al. (2011) and Mittal & Griskevicius, (2014), who measured housing conditions and financial difficulties, respectively—but worth noting is those studies also included measures of income and parental education.

To conclude this section: The contrast in findings between Jovanovic et al. (2017), Peng et al. (2019), Chen et al. (2016), Wickline et al. (2011); Ahlin, 2014, Mittal & Griskevicius, (2014), and Pedron et al. (2021) and the current study may suggest that the type of poverty indicator matters. The null findings in the current study suggest that independently, or together as a cumulative measure, financial difficulties and neighborhood stress are not sufficient indicators of poverty to predict epigenetic age or LoC. Replicating the current research but using measures of parental education and income rather than financial difficulties and neighborhood stress may result in different findings.
The Type of Epigenetic Age Calculator May Matter

Another potential reason that the current study may have found null results—and perhaps a cautionary note for future research using the Horvath Clock—may have something to do with the epigenetic age measurement tool itself. The Horvath Clock was a valid DNA methylation clock to use for the present study because it is a pan tissue clock, that is, it can be used confidently with DNA from any tissue. Further, since it was developed using DNA from cohorts ranging from birth through centenarians, it is valid for any age group, including children (Horvath, 2013). With that noted, however, it is possible that the Horvath Clock was a poor assessment tool for epigenetic age during childhood. While the Horvath Clock can be used to assess epigenetic age at age 7, it has been used in a relatively few pediatric populations (see Wang & Zhou 2021 for a summary of epigenetic clocks used in pediatric populations). Several notable studies—those that examined a pediatric population with the Horvath Clock—are worth discussing.

While Jovanovic et al. (2017) and Sumner et al. (2019) utilized the Horvath Clock for childhood epigenetic age analysis, their samples included participants that were not only children (i.e. 6 or 8 years old), but also teenagers—up to 13 and 16 years old. While these reports demonstrate significant associations between adversity and epigenetic age as calculated by the Horvath Clock, their sample included a wider range, and an older, population of participants. Further, as an exemplar of the lack of associations between the Horvath Clock and outcomes in pediatric populations, consider Simpkin et al. (2016), who evaluated numerous potential correlates of epigenetic age during childhood within the ARIES cohort. Their extensive analysis only yielded four significant associations: birthweight, maternal BMI, maternal cholesterol, and levels of cotinine.
To reiterate, while the Horvath Clock is a valid measure to assess epigenetic age in childhood, relatively few studies have reported its use in pediatric populations. The reason, however, for the inconsistent use of the Horvath Clock in pediatric populations could be due, in part, to the lack of associations it has with characteristics from that age population. Along the same lines, recently it has been reported that the Horvath Clock lacked precision with chronological age predication in pediatric cohorts (McEwen et al., 2020). With the potential limitations to the Horvath Clock for pediatric populations, other tools and methods to assess epigenetic age in pediatric populations are worth considering—for example, using multiple or age-specific DNAm clocks to evaluate a cohort. These methods are discussed next.

In contrast to the current study, a trend in epigenetic age literature is to evaluate epigenetic age using a variety of different DNAm clocks. Including multiple epigenetic clocks, perhaps, is due diligence or an act of methodological thoroughness. A more pessimistic point of view, however, is that certain epigenetic clocks result in null findings, so others are “sampled” until a significant association arises. In the absence of multiple test corrections, this practice could lead to non-replicable findings.

Regardless of the rationale to use multiple epigenetic clocks within a study, it is not uncommon that only one epigenetic clock results in a significant association. To highlight this phenomenon, consider Marini et al. (2020), who presented compelling evidence regarding childhood adversity during sensitive windows influencing epigenetic age at age 7 within the ARIES cohort. Marini and colleagues (2020) reported that financial hardship, neighborhood disadvantage, and abuse were found to be independently associated with epigenetic age at age 7. Marini et al. (2020), in other words, found associations between the same indicators of poverty and epigenetic age at the same timepoint that the present study evaluated. The present study,
however, found null associations. The reported the associations of childhood adversity and epigenetic age by Marini et al. (2020), however, were determined by an epigenetic clock called the Hannum Clock. That is, Marini and colleagues (2020) evaluated several epigenetic age clocks (i.e., Horvath Clock and Hannum Clock) and found that the Hannum Clock resulted in significant associations while Horvath Clock did not. This finding might be explained by the fact that the Horvath Clock was developed using a variety of tissues while the Hannum clock was developed using only blood (Marini et al., 2020). Another explanation—discussed further next—might involve the age of participants used to develop each DNAm clock.

The validity of the associations presented by Marini et al. (2020) are in question because the Hannum clock was not developed (i.e. trained) on pediatric populations. In other words, the machine learning technique used to develop the Hannum Clock did not “teach” the regression equation to consider DNA from children, thus its application to pediatric populations is perhaps inappropriate. Regardless of the contrast in findings and the question of validity, future research could replicate the current study with multiple epigenetic clocks or, more broadly, future research could elucidate the discrepancies in epigenetic clocks used in the same population.

Another trend in epigenetic age literature is the use of DNAm clocks developed for a specific population. One such DNAm clock is the PedBE Clock. The PedBE Clock was created using pediatric data sets (i.e., ages 0-20), and was specifically designed to evaluate the epigenetic age of children (McEwen et al., 2020). Since it was designed using pediatric cohorts, the PedBE Clock seems to have greater accuracy—that is, less predictive error—in predicting chronological age in pediatric samples compared the Horvath Clock (McEwen et al., 2020). Recently, using the PedBE DNAm clock, associations between internalizing symptoms and maltreatment were reported in young children (Dammering et al., 2021). Re-evaluating the model in the current
research with the PedBE Clock might yield different insight into the relationship between childhood adversity and epigenetic age.

**Epigenetic Age May Not Be Related to Locus of Control**

The current study found that epigenetic age is not associated with locus of control and supports the notion that epigenetic age is not associated with cognition. Results of the current study parallel those reported by Suarez et al. (2018) who also did not find associations between epigenetic age and cognition.

The hypothesized association between epigenetic age and LoC was plausible drawing on evidence that epigenetic age is associated with pathologies of cognition like internalizing behaviors (Suarez et al., 2018; Tollenaar et al., 2021) and depression (Suarez et al., 2018). The influence of epigenetic age on LoC, however, cannot be ruled out. It may be the case that epigenetic age has a distal influence on LoC. With that noted, more proximate influences are worth considering, like changes to brain structures.

Considering that locus of control is a facet of cognition that occurs in the brain, it makes sense to examine biological mechanisms pertaining to changes in the brain. As noted in the Introduction, there is evidence that childhood adversity is associated with changes to brain structures (Heim et al., 2008; Teicher et al., 2012). For example, Colich et al. (2020) reported that early life adversity, deprivation, and SES were correlated with cortical thinning.

Further, changes to the brain are a plausible mechanism to evaluate because there is evidence that LoC is associated with changes to brain structures. For instance, LoC was found to be associated with hippocampal volume (Pruessner et al., 2005) and gray and white matter volume (Hashimoto et al., 2015). With this argument in mind, future research might investigate changes to brain structures as a proximate mechanism linking poverty and LoC.
Males May Have a Higher Epigenetic Age and Females May Be More Sensitive to Adversity

Males and females have different epigenetic ages (Brody et al., 2016a; Sumner et al., 2019) and within this cohort it has been reported that females have a lower epigenetic age than males (Simpkin et al., 2016). Male and females also have different LoC orientations (Awaworyi Churchill et al., 2020). Thus, it was hypothesized that gender stratified analyses might reveal gender specific relationships. Gender stratified analyses of the mediation model, however, remained insignificant. In other words, there was no evidence that epigenetic age mediated the relationship between poverty and locus of control when males and females were evaluated independently.

There were no group differences in LoC orientation or poverty experienced. Males, however, had a slightly higher epigenetic age than females\(^3\). The current study suggests, perhaps, that this gender difference in epigenetic age is not due to differences in amounts of poverty because although males had an older epigenetic age, the amount of poverty did not differ between the groups.

Further, poverty, epigenetic age, and locus of control were not correlated within males. Within females, however, while other correlations were not significant, one association—that between poverty and epigenetic age—was borderline. Females that experienced higher levels of poverty tended to have higher epigenetic ages. This may indicate that females are more sensitive—reflected in their epigenetic age—to childhood poverty. One may argue that the correlation failed to reach the .05 threshold, therefore the inference drawn is useless. Consider,

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\(^3\) While the current study found that the difference in epigenetic age between females and males was significant, Simpkin et al. (2016)—analyzing the same data—did not. With that noted, however, the current study and Simpkin et al., (2016) both found that males had higher epigenetic age—Simpkin and colleagues (2016) first noting the difference in adolescence. The discrepancy in results may be due to outlier elimination in the current study as when the analysis was re-run with the excluded outliers included, the gender difference in epigenetic age was null. This demonstrates the “borderline” nature of this relationship and why it should be treated cautiously.
however, that this positive relationship is opposite to the trend of epigenetic age gender differences—that is, within the cohort, females typically have a lower epigenetic age. This observation may make this borderline finding indicative of a real change.

Another argument can be made that poverty began to influence the epigenetic age of the female participants and this study captured the “start” of a developmental trajectory marked by advanced epigenetic age and initiated by adversity. This would mean that the influence of adversity is minimal in early childhood and increases with age. That is, as the females get older, the epigenetic age of those experiencing adversity would continue to climb. That gender differences in epigenetic age are minimally present early but develop over time as a result of adversity is evidenced by Tang et al. (2020). Tang and colleagues (2020), also evaluating the ARIES cohort, reported gender differences and associations between childhood adversity and epigenetic age within female participants age 17. Specifically, cumulative ACE score, physical abuse and emotional abuse were all associated with epigenetic age $p < .05$.

The observation that the influence of adversity on epigenetic age in females develops over time does not explain why the epigenetic age gender differences develop in the ARIES cohort. That is, what could explain the epigenetic age divergence beginning in childhood and fully manifesting in adolescence?

First, one may infer that the females in the current study and the females in the Tang et al. (2020) study experienced more adversity. That is, however, not the case—as discussed already—males and females experienced the same amount of adversity in both. Specifically, as it pertains to the present study, males and females with the ARIES cohort did not differ in the amount of poverty experienced $t(892) = -1.312, p > .05$. 
Second, in line with the series of arguments outlined earlier regarding severity, the inference can be made that while males and females experience the same amount of adversity, a greater percentage of girls experienced an adversity considered severe—especially as they aged. This inference cannot be addressed with the current study since it only investigated poverty at age 7, although this would be an interesting line of inquiry. That female participants experienced more severe adversities is, in fact, what was observed in Tang et al. (2020)—a greater percentage of females experienced sexual abuse than males. The epigenetic age gender difference, therefore, may be due to females experiencing more severe adversities than males as they age.

Third, the divergence in epigenetic age between males and females may be due to a difference in stress sensitivity. Consider the argument discussed earlier regarding the changes in DNAm as a function of adversity severity. With higher levels of adversity (i.e. stress), the DNAm levels of the CpGs regulating the stress response that also comprise the Horvath Clock change which may result in a shift in epigenetic age. Interestingly, Tang et al. (2020) also measured cortisol levels and report a gender difference: Females had significantly higher levels of cortisol than males. With respect to the current study, though both male and female children experience the same amount poverty, perhaps the female children perceived the poverty as more adverse (i.e., stressful) resulting in an increase in epigenetic age. Future research could mimic the report by Tang et al. (2020) and untangle these inferences by examining cortisol levels within the children at age 7.

**Strengths and Limitations of The Current Study**

The first strength of the current study is the large sample size. Many existing reports that investigated the relationship between ACEs and epigenetic age used small sample sizes (e.g., $N =$
101, Jovanovic et al., 2017; \( N = 247 \), Sumner et al., 2019). The current study analyzed 894 participants.

Second, while the design of the research was a secondary data analysis, the data is prospective. This is particularly an important methodological component with reference to ACEs because retrospective ACE reports are potentially biased (Green et al., 2010; Hardt & Rutter, 2004). The prospective design of this research may have improved the validity of the ACE measures.

The final strength of this research is that it utilized a source of data that adequately addressed issues typically associated with SDA. For example, two common SDA issues are (a) the original data was collected to answer a different research question and thus (b) the variables in the proposed research are not addressed with adequate breadth and depth. As discussed, the ALSPAC cohort was created to address questions like the one asked by this research. That is, the ALSPAC cohort is poised to address how environmental factors influence epigenetic patterns that modify developmental trajectories. Further, the ALSPAC cohort contains various measures of poverty assessed longitudinally, assessed LoC using 12 items, and contains epigenetic data on over 1,000 participants.

**Limitations**

With the strengths of this research noted, there are, however, several limitations. First, while the merits of poverty as an ACE independent variable have been discussed and justified, the current research lacked a complete ACE profile. In other words, while poverty is comorbid with other ACEs (Evans & Kim, 2013; Walsh et al., 2019), many reports that investigate the same relationships utilized a more heterogeneous ACE variable profile (e.g., Marini et al., 2020; Tang et al., 2020). Future research can address this deficiency in two ways. First, add either more
poverty measures if the goal is to assess poverty and second, include different types of ACEs—perhaps of greater severity—to create a more scoping ACE profile.

The second limitation is that the results of the mediation analysis must be discussed in light of low locus of control item reliability. While the low item reliability has been addressed and the locus of control variable deemed appropriate for use, the items on the shortened version of the CNSIE, nonetheless, exhibited less than satisfactory reliability within the ARIES cohort.

A final limitation involves the demographic profile of the ARIES cohort and the ALSPAC data set as a whole. The results and inferences drawn from the current study are not generalizable because the sample was almost entirely Caucasian.

Conclusion

This secondary data analysis showed that epigenetic age did not mediate the relationship between poverty and locus of control. There was no evidence that poverty got under the skin through modifications to DNAm as measured by the Horvath Clock. While the findings in the current study were almost entirely null, there are several important inferences that can be drawn and can be summed as follows.

First, we may have learned that the severity of childhood adversity matters. Abuse and other forms of violence may have a more immediate influence on both epigenetic age and LoC than poverty. Second, we have learned that indicators of poverty may influence epigenetic age and LoC; the associations, however, may develop and strengthen with time. That is, the effect of poverty on epigenetic age and LoC may begin in childhood but have a measurable effect in adolescence. Third, we have learned that not all poverty indicators are created equal. This study, in conjunction with other studies, may demonstrate that income and parental education, as indicators of poverty, are more salient variables when evaluating poverty. Fourth, this study is
evidence that measures of epigenetic age are idiosyncratic and the type of epigenetic clock is an important consideration prior to an investigation. Fifth, we may have learned that other biological mechanisms—like brain-based mechanisms—are worth considering as this study provides evidence that epigenetic age is not a biological mechanism connecting childhood adversity with LoC. Last, we may have learned that females are more sensitive to adversity in childhood.

The current research not only adds to existing literature the aforementioned inferences, but also informs future research—that is, the current study can be replicated with several changes. For example, the current study justifies the evaluation of severe adversities in childhood and other indicators of poverty, like income and parental education. Moreover, the results from the current study suggest that other epigenetic age calculators are worth considering. Finally, further research can explore other mechanisms connecting adversity in childhood with facets of cognition, like brain structures and stress sensitivity.


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http://www.bristol.ac.uk/alspac/researchers/our-data/questionnaires/carer-questionnaires/

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Appendices

Appendix A: Neighborhood Stress, Financial Difficulties, and CNSIE Items

*Neighborhood Stress Items*\(^a\)

Here is a list of some things that can be a problem in people's homes or in the neighborhood. How much of a problem are the following for you and your family?

<table>
<thead>
<tr>
<th>Item</th>
<th>Serious Problem</th>
<th>Minor Problem</th>
<th>Not a Problem</th>
<th>No Opinion</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Badly fitted doors and windows</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b) Poor ventilation</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>c) Noise travelling between the rooms of your home</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d) Noise from other homes</td>
<td></td>
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</tr>
<tr>
<td>e) Noise from outside in the street</td>
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<tr>
<td>f) Rubbish or litter dumped around your neighborhood</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g) Dog dirt on pavements/walkways</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>h) Worry about vandalism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) Worry about burglaries</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>j) Worry about muggings or attacks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k) Disturbance from teenagers or youths</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Items retrieved from [http://www.bristol.ac.uk/alspac/researchers/our-data/questionnaires/carer-questionnaires/questionnaire database](http://www.bristol.ac.uk/alspac/researchers/our-data/questionnaires/carer-questionnaires/questionnaire database)
Financial Difficulty Items\textsuperscript{a}

How difficult at the moment do you find it to afford these items:

<table>
<thead>
<tr>
<th>Item</th>
<th>Very Difficult</th>
<th>Fairly Difficult</th>
<th>Slightly Difficult</th>
<th>Not Difficult</th>
<th>Don’t Pay For This</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clothing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rent or mortgage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Things you need for your child</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Costs of educational courses (e.g., ballet, music, etc.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Medical or dental care</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>*Child care</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>*Something else</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Denotes an item found only on the assessment administered at 85 months

\textsuperscript{a} Items retrieved from [http://www.bristol.ac.uk/alspac/researchers/our-data/questionnaires/carer-questionnaires/](http://www.bristol.ac.uk/alspac/researchers/our-data/questionnaires/carer-questionnaires/) questionnaire database
Locus of Control: Shortened version of the Nowicki-Strickland Internal-External scale

- Wishing makes good things happen
- People are nice no matter what you do
- Usually do bad at schoolwork
- Friend angry, hard to make like again
- Surprised when praised by teacher
- Bad things happen, someone else’s fault
- Doing will in class is a matter of luck
- Often blamed for things
- Argument/fight other person’s fault
- Preparing for tests is a waste of time
- Nice things happen due to luck
- Planning ahead makes good things happen
Appendix B
Epigenetic data methods and quality control procedures

To collect the epigenetic data, blood was first collected, and the DNA was subsequently bisulfite converted using the Zymo EZ DNA Methylation TM kit (Zymo, Irvine, CA). The Illumina Infinium HumanMethylation450k BeadChip (Illumina Inc., CA) was used to profile and quantify the DNA methylation patterns. The arrays were scanned using the Illumina iScan. GenomeStudio (version 2011.1) was used to perform an initial quality review (Relton et al., 2015).

The full quality control protocol is described elsewhere (Relton et al., 2015). Briefly, several steps were taken to ensure that samples were removed due to issues such as batch effects and sample mismatches. For example, samples were semi-randomly distributed across the arrays to minimize the confounding influence of batch effects. Further, each array has quality control probes whose metrics are reported. Any slides failing to meet quality control (p >= .01) were discarded and excluded from any further analysis. Lastly, to identify sample mismatches, genotype data were compared to SNP data from the assay and samples were checked based on X chromosome methylation (Relton et al., 2015).

The methylation data were pre-processed and quantile normalization was performed. Post hoc cell heterogeneity correction procedures were performed using the estimatecellcountes function in the minfi Bioconductor package in R (Relton et al., 2015) because white blood cell heterogeneity is known to confound DNA methylation measurements (Jaffe & Irizarry, 2014; Reinius et al., 2012).