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Exploring Genetic Moderators and Epigenetic Mediators of Contextual and Family Effects: From G×E to Epigenetics

Steven R. H. Beach,

Center for Family Research, University of Georgia

Gene H. Brody,

Center for Family Research, University of Georgia

Allen W. Barton, and

Center for Family Research, University of Georgia

Robert A. Philibert

University of Iowa

Abstract

In the current manuscript we provide an overview of a research program at the University of Georgia's Center for Family Research designed to expand upon rapid and ongoing developments in the fields of genetics and epigenetics. By placing those developments in the context of translational research on family and community determinants of health and well-being among rural African Americans we hope to identify novel, modifiable environments and biological processes. In the first section of the article, we review our earlier work on genotypic variation effects on the association between family context and mental and physical health outcomes as well as differential response to family-based intervention. We then transition to discuss our more recent research on the association of family and community environments with epigenetic processes. In this second section of the article, we begin by briefly reviewing terminology and basic considerations before describing evidence that early environments may influence epigenetic motifs that potentially serve as mediators of long-term effects of early family and community environments on longer-term health outcomes. We also provide evidence that genotype may sometimes lead to Gene × Environment (G×E) effects. Finally, we describe our recent efforts to use genomewide characterization of epigenetic patterns to better understand the biological impact of protective parenting on long-term shifts in inflammatory processes and its potential implications for young adult health. As will be clear, research on epigenetics as a mediator of the connections between family/community processes and a range of health outcomes is still in its infancy, but the potential to develop important insights regarding mechanisms linking modifiable environments to biological processes and long-term health outcomes already is coming into view.

Contextual and Family Effects on Health and Well-being: From G×E to Epigenetics

Ten years ago scholars at the University of Georgia's Center for Family Research (CFR) began contemplating the ongoing genomic revolution, its implications for family science, and its potential to lead to advances in family intervention. Marking the completion of the sequencing of the human genome, Francis Collins and colleagues (2003) had announced that "The genomic era is now a reality," and several observations became immediately apparent. First, it seemed apparent that robust developments in molecular genetics were bringing the assessment of genetic variation within reach of family scientists and would allow the use of genotyping as a routine research tool. Second, persuasive and well-replicated results in behavioral genetics suggested that very soon there would be a number of well-replicated sets of genetic variants with additive main effects that could be related to outcomes of interest to family researchers in the form of genetic risk indices (cf. Plomin, Owen, & McGuffin, 1994). We assumed that these forthcoming genetic indices would account for substantial variance in outcomes and so help refine our understanding of family effects on these same outcomes, and that family processes would be understood as amplifiers of genetic effects or mediators of genetic main effects on outcomes. Accordingly, it also seemed likely that continued developments would make genotyping available as a routine clinical tool, providing genetic risk information to guide intervention. Finally, we assumed that the genetic revolution in our understanding of health, health behavior and related family outcomes was here to stay.

We were correct in two of our three assumptions. As we anticipated, the cost of genotyping has dropped substantially and rapidly over time (see for example <http://www.genome.gov/sequencingcosts/>), and the rapid expansion of information about genetic contributions to behavior has continued to expand unabated. However, in contrast to our expectations for the quick emergence of useful genetic indices, indices capturing additive main effects on outcomes of potential interest have only recently begun to emerge.

The relative slowness of progress with regard to development of genetic indices of risk sparked controversy about the so-called problem of "missing heritability," referring to the discrepancy between variance in outcomes shown to be associated with molecular variants relative to expectations based on behavioral genetic models (Van IJzendoorn, et al., 2011). In response to these issues, as well as widely noted conceptual developments based on evolutionary considerations (Ellis et al., 2011), there was an upsurge in interest in identifying "sensitivity or susceptibility" genes as another way to reconcile molecular genetic findings with heritability estimates. Research at the Center for Family Research on Gene × Environment (G×E) and Gene × Intervention (G×I) effects fits within this later trend toward identifying potential subgroups who are more responsive than others to the impact of both environmental context and intervention.

As the genetic revolution expanded, it gave rise to interest in epigenetic assessment, identifying another layer of genetic influence and control. Of particular interest to prevention researchers, epigenetic change appeared to have the potential for being responsive to manipulated environments, suggesting it might capture processes of direct

translational interest. Again, the increasing availability and affordability of assessments designed to characterize epigenetic modifications at ever increasing scales led us and other family scientists to wonder about the possibility of investigating the impact of family and other environments on this crucial layer of genetic regulation. Much of our work at the Center for Family Research on epigenetics examines epigenetic indices as potential mediators or moderators of family and community effects on health (but see also the manuscript by Brody in this volume, and work by Philibert, Beach, & Brody, 2012 that illustrates the value of epigenetic indices as outcomes in their own right). Because our genetic and epigenetic research efforts are linked historically and conceptually we summarize both in the following article. We begin by presenting an overview of our research examining genetic effects on affective, behavioral, and family outcomes; then, in the second half of the article, we orient the reader to our current efforts to probe the association of family environments with epigenetic change and the way these changes may be related to longer-term health and health-behavior outcomes.

Background

For twenty years the Center for Family Research has focused on identifying protective processes for rural African American youth. Utilizing Institute of Medicine (IOM) guidelines to inform our work, we first develop empirically supported etiological models and then use these models to guide the development of preventative interventions. In response to accumulating and consistent evidence from behavioral genetic paradigms indicating strong “heritability” for many health and behavioral health problems, it was natural for us to begin integrating molecular genetic data into our ongoing basic and prevention research projects. Of particular interest was the possibility that controlling for genetic influences on outcomes might better illuminate the variance attributable to the family and community protective processes that shield youth from economic and social adversity due to resource poor rural environments. Specifically, adding genetic data was expected to increase the power of our etiological models and lead to new insights that could be translated into family centered preventative interventions, interventions which in turn, would be evaluated in randomized prevention trials (e.g., Brody et al, 2012).

In keeping with our ongoing collaboration with communities and families, prior to collecting genetic data, we convened focus groups of African American caregivers and youth to discuss this shift in direction by CFR and elaborate potential concerns. Collection and utilization of genetic data has the potential to be of concern for any group that has suffered exclusion or other forms of discrimination, and such concerns could overwhelm potential benefit. Based on participant feedback, brochures were designed to address participants’ concerns and provide them with clear, culturally relevant information. The recruitment protocols that were developed have yielded agreement rates averaging 90% of participants contacted across projects. This high rate of agreement is important because low participation reduces both power to detect effects and confidence in any obtained G-E interplay effects, underscoring that good community engagement and trust is essential for good prevention science.

Candidate Genes

Our initial research efforts focused on explicating the way in which genetic variation influenced response to the social environment, developmental context, and/or preventive interventions, and focused primarily on just two candidate genes: the serotonin transporter (*5HTT*) and the dopamine receptor (*DRD4*). We focused on these candidate genes as both had a long history in the literature on G×E effects and had emerged as prominent members of the broader set of “susceptibility” genes identified by leading researchers in this area (e.g., Belsky & Pluess, 2009; Ellis et al., 2011).

The serotonin transporter gene, (SCL6A4), was a natural focus of initial interest for the CFR given the potential influence of serotonergic systems on mood regulation and other health and health behavior outcomes. The serotonin transporter protein is important in serotonergic activity in the central nervous system and throughout the body in that it regulates the reuptake of serotonin following synaptic release. The most commonly studied polymorphism in *SLC6A4* is in its highly polymorphic promoter region known as the “5-HTTLPR,” a polymorphism that results in two common variants, a long (*l*) and a short (*s*) allele, as well as some less common variants (e.g., very long (*vl*)). The *s* variant is associated with lower availability of 5-HTT and reduced efficiency of 5-HTT reuptake. Of interest to family prevention researchers, carrying a short-variant of the polymorphism has been found to be associated with alcohol consumption in multiple samples (e.g. Kaufman, Yang, Douglas-Palumberi, 2007; Munafò, Lingford-Hughes, Johnstone, Walton, 2005).

Likewise, the gene encoding the *dopamine receptor D₄ (DRD4)* was a natural focus of attention due to its important role in regulating the neurotransmission of dopamine, a chemical implicated in the reward and pleasure centers of the body. Like the serotonin transporter gene described above, there is a highly polymorphic region on *DRD4* that is characterized as a variable nucleotide repeat, but unlike *SLC6A4*, the polymorphic region on *DRD4* is in the coding area of the gene and so results in changes in the structure of the protein itself, not just in the quantity of protein produced. The *DRD4* VNTR contains 2 to 11 repeats with the 4-repeat and the 7-repeat alleles being most common. Youths carrying at least one 7-repeat allele have been found to engage in greater alcohol use (Conner, Helleman, Ritchie, Noble, et al., 2010; Laucht, Becker, Blomeyer, & Schmidt, 2007) than similar youth without the allele. Again, although the literature is not entirely consistent, the preponderance of studies suggest that the 7-repeat allele variant of *DRD4* forecasts increases in youths’ problem behavior in response to variations in parenting (e.g. Bakermans-Kranenberg, et al., 2006, 2007, 2011).

An alternative approach to examine candidate genes might have been to conduct genomewide association studies (GWAS). The prominence and potential availability of genome wide platforms continues to grow, and there are well-documented racial differences in variability across the genome that support the likely importance of separate GWAS focused on African Americans. Nonetheless, there are two main reasons why we did not attempt to do our own GWAS to identify promising candidate variation in genes, or to construct our own genetic indices that might be particularly relevant for African Americans. First, as a practical consideration, we have been underpowered to correct for the number of comparisons that are typical of genome wide approaches. Correction for a very large number

of comparisons requires very small nominal p-values, and so calls for much larger samples than we have typically utilized in our research. This problem is amplified for researchers, like those at CFR, who also are interested in examination of G×E or other gene-environment transaction effects. Examining potential interaction effects multiplies the number of effects to be examined, increasing the needed sample size even further. This is a problem likely to be shared by most family-oriented prevention researchers. As briefly noted above, we also have not utilized GWAS-derived genetic indices derived by others despite our initial intention to do so because genome wide approaches have substantially underperformed relative to expectations. Indeed, while some common genetic variants associated with outcomes and traits have been identified, they typically account for only a small proportion of heritable trait variance (e.g., Weedon, et al., 2008), much less than expected. This problem, often referred to as the “missing heritability problem,” has been attributed to multiple causes (see van Ijzendoorn et al., 2011), such as the possible lack of attention to G×E effects, the relative inability of genome wide approaches to accurately characterize the influence of variable repeat motifs in the genome, a failure to accurately characterize the cumulative impact of very small additive effects from numerous genetic sources, or perhaps biases in behavioral genetic approaches that may overestimate heritability in some cases.

We now turn attention to summarizing selected studies conducted by our research center into the genetic susceptibility effects of 5HTT and DRD4 for G×E and G×I hypotheses.

Testing Etiological Models

An examination of the effect of involved-supportive parenting in interaction with 5HTT—In one early investigation we used a prospective design to investigate whether carrying an *s* allele at the 5-HTTLPR increased vulnerability to substance use among African American youths from age 11 to age 14 years (Brody, Beach, Philibert, et al., 2009). We predicted that variability in the 5-HTTLPR would be associated with the growth of substance use from age 11–14, and that involved-supportive parenting would attenuate the link between variation at 5-HTTLPR and increases in substance use across late childhood and early adolescence. Drawing on prior research, we characterized involved-supportive parenting during this developmental period as including high levels of emotional support, instrumental assistance, and communication, i.e., parenting practices that have been shown to be protective for African American youth and to reduce the impact of other risk factors on youth substance use. The results indicated that, as expected, presence of the *s*-allele was linked to the development of substance use. Among those youth with lower levels of supportive parenting, the link was particularly clear, but the presence of the *s* allele was not a risk factor for the development of substance use when youths received high levels of involved-supportive parenting. The risk conferred by 5-HTTLPR status on youths whose parents engaged in low levels of involved-supportive parenting was three times as large as the coefficient among youths whose caregivers provided high levels of such parenting.

Subsequent longitudinal research that included variability at the 5-HTTLPR as a moderator replicated and extended these findings (e.g. See Brody et al., 2011; 201; Kogan et al., 2010), suggesting that G×E effects could be identified, potentially implicating different pathways to substance use and other negative outcomes. These findings supported examination of *s*-allele

effects in the more rigorous context of intervention research (i.e., G×I), a context in which the environment is experimentally manipulated, allowing stronger conclusions about both environmental and genetic moderation. However, before reviewing our G×I research with 5-HTTLPR, we first briefly review our etiological research on phenotypic variation associated with individual differences in *DRD4*. We will then review our efforts to use preventive intervention as a way to better examine G×E effects at both *5HTT* and *DRD4*.

An examination of resilience, diathesis-stress, and differential susceptibility effects involving *DRD4*—As for other putative genetic vulnerabilities, the processes hypothesized to protect youth from the vulnerability (or susceptibility) conferred by *DRD4* includes the quality of a range of contextual processes such as the parenting they receive, their connection and commitment to schooling, and the type of peers with whom they affiliate. As this list suggests, some variants of *DRD4*, particularly longer VNTRs, appear to increase the impact and importance of positive structure and social engagement for long-term positive outcomes. Thus, we hypothesized (Brody et al., 2012) that youth with *DRD4* variants of 7R or longer would be more susceptible to substance use increase during adolescence. Results indicated that, as expected, *DRD4* was linked to the development of substance use across adolescence, and this association was moderated by high levels of involved-supportive parenting, high levels of deviant peer affiliations, and both high and low levels of school engagement (Brody et al., 2012).

These initial forays into the examination of G×E effects were promising, but raised the question of whether such effects were due, in part, to unmeasured third variables, gene-environment correlations, population stratification, or other potential sources of spurious relationships. Although we took steps to examine and eliminate these potential problems statistically in our longitudinal research, an alternative, and more powerful approach was also available to us: the use of prevention trials to more rigorously test causal hypotheses by using experimental design to eliminate some competing hypotheses.

Testing Genetic Effects on Intervention Response

One potential problem with observational research is the possibility that in some cases genotype may correlate with family or community environments, posing interpretative problems. Such gene-environment correlations are commonly referred to as rGE effects (see Rutter, Moffit, & Caspi, 2006) and can arise from several different causes. To avoid this problem and more rigorously examine environmental effects we turned to randomized, controlled prevention trials. Through the implementation of experimental designs, a causal relationship between an environmental manipulation and the alteration of a targeted outcome can be identified, ruling out rGE as a rival explanation and providing a firmer foundation for identification of pure environmental effects. In addition, randomized experiments tend to have greater statistical power than correlational designs; consequently, fewer participants may be needed to reliably detect a G×E interaction in a randomized trial or other experimental design relative to longitudinal or other correlational designs (McClellan & Judd, 1993).

Using randomized prevention trial designs to examine the interaction of family processes with *5HTT* and *DRD4*—To examine the moderating effect of *5HTT* and *DRD4*, we used data from randomized prevention trials in which manipulated family processes had been shown to be causal factors. For example, Brody et al. (2009) found that the Strong African American Families (SAAF) intervention protected against substance use initiation among children carrying one or two copies of the serotonin transporter short allele at the 5-HTTLPR. Similarly, Beach, Brody, Lei, et al. (2010) demonstrated that preadolescents who carried the 7-repeat version of *DRD4* and were assigned to take part SAAF had less substance use across two years than did youths with the same genotype who were assigned to the control group. Likewise, Brody et al. (2012) found that the Adults in the Making (AIM) Program, a family-centered substance use prevention program for 17-year-old African Americans, moderated the impact of *DRD4* on reductions in substance use. Specifically, adolescents living in high-risk families who carried at least one *l* allele of *DRD4* and who were assigned to the control condition had more rapid increase in drug use than did (a) adolescents who lived in high-risk families who carried at least one *l* allele of *DRD4* and who were assigned to AIM or (b) adolescents assigned to either condition who carried no *l* alleles. A recent follow-up to this study indicates that Aim induced reductions in vulnerability cognitions accounted for the Family Risk x AIM x *DRD4* interaction effect on substance use increases (Brody, Yu, Beach, 2015), illustrating the way in which GxI studies can help illuminate mechanisms of change. These studies provide experimental support for the importance of GxE effects on the impact of family environment while suggesting etiological hypotheses.

Additional studies have continued in this tradition, helping clarify neighborhood risk factors (e.g. Windle et al, 2015) and potential mechanisms linking stress to increased vulnerability to substance use (e.g., Brody et al., 2012). In addition, this work has inspired development of new designs and analytic approaches for genetically informed research (e.g., Howe, et al., in press). These studies suggest that there is considerable merit in using prevention trials to provide a careful examination of genetic effects, and that prevention trials may be particularly useful in the examination of possible GxE effects. These studies also add to the growing body of research suggesting that genetic moderation may help identify variations in the impact of family processes on particular individuals, strengthening etiological models. It seems likely that these findings will prove even more compelling as multi-gene indices are developed and utilized to illuminate more powerful relationships (e.g. Brody et al., 2014).

Additional Considerations—There also have been some initial efforts to examine family-systems level effects of genetic variation, adding some potential complexity to GxE and GxI models. For example, it seems likely that differential response by one family member has the potential to ripple through the family system, affecting outcomes for themselves and other family members while influencing family processes. This potential “systemic” effect of genotype was initially demonstrated in research by Beach et al. (2009). They found that youths’ genetic status (i.e., having a short allele on *5HTT*), moderated mother’s response to a parenting intervention, with depressed mothers whose offspring had the short *5HTT* allele showing greater relief from their own (i.e., the mothers’) depression after participation in an intervention program focused on helping youth. Indeed, youth

genotype, and not mother's genotype, moderated mothers' response. A "family-systems" effect also has been reported for *DRD4*. Beach et al (2012), found that stressors external to the family – such as community level stressors - were more likely to be amplified and turned into stressors within the family (i.e. by increasing negative family interactions) when the parent was a carrier of the *DRD4* long allele and experienced greater external stress (see also Cho & Kogan, 2015).

Accordingly, it seems increasingly likely that family and systemic models describing etiology and treatment effects will incorporate genetic factors in various ways (see also Brody et al., 2013). Taken together, this literature again supports the potential utility of examining variable repeat motifs as an important source of human genetic variability and suggests that those at highest genetic "risk" or with the greatest genetic "sensitivity" may be most likely to benefit from behavioral or environmental interventions. As genetic risk indices and measures that better capture the activity of gene networks become available, capturing greater portions of variance in key mediators and outcomes, it seems likely that these initial effects will be extended. Likewise, when whole genome sequencing approaches become affordable and are more widely utilized it may be possible to better identify the impact of additional low-frequency genetic variation. However, even given the results obtained to date, there is a practical question to address. Should we begin to use genotypic information to guide preventive intervention? For many this would seem to be a natural next-step after identifying significant GxI interaction effects. But, even setting aside the preliminary nature of the results obtained to date, there are reasons to postpone such a step, and perhaps to question whether it is ever likely to be preferable to use genetic indices in place of family history or phenotypic information when considering indicated prevention programs.

Why not use genotype data to efficiently guide preventive intervention?—

Although the argument for genetic selection of participants in preventive intervention can be tempting, we believe targeting interventions based upon genotype is problematic for several reasons including 1) the prevention paradox, 2) the problem of unintended negative effects of selection on both those selected and those not selected, and 3) the practical and conceptual problems resulting from the identification of an increasing number of "susceptibility" alleles, i.e., the *problem of success*. Each of these potential concerns is relevant to the data already in hand, and is likely to remain pertinent even as more comprehensive and more powerful genetic indices become available.

The Prevention Paradox refers to the observation that the majority of cases of most complex disorders come from parts of the population that would be defined as being at low to moderate risk for that disorder, with only a minority of cases typically coming from the highest-risk portion of the population (Rose, 1981). This is a consequence of highest-risk individuals being less frequent overall. So, even though they contribute more cases proportionally, they still do not contribute the majority of cases overall. A developmental example of this concern is that even if "early starters" are of particular interest due to their elevated long-term risk of problems, they are unlikely to comprise a majority of those with substance use, health, or other health behavior problems in young adulthood or middle age. As a consequence, selectively offering a preventive intervention only to those individuals at

highest risk (or risk for earlier onset) may effectively and efficiently reduce the individual risk of those participating in the program but still be relatively ineffective at reducing the burden of total prevalence of the disorder in the population.

Potential for Discrimination and Stigmatization of those selected may occur when selected targets or their families are treated differently on the basis of their high-risk status (Offord, et al., 1998; Robson, et al., 2010). This is a particular concern in populations that may have suffered previously from discrimination, potentially setting the stage for a prevention program to activate pre-existing concerns. Also, in the context of genetic screening, even asymptomatic individuals or their family members may be perceived or treated differently due to their real or presumed genotype. That is, they may believe they are being viewed or treated as “problematic” despite not having the disorder or problem themselves. Likewise, *negative effects on the unselected* portion of the population can occur if unintended messages are sent during the selection process. For example, children or parents not selected for prevention programming may conclude that they must be relatively “invulnerable” to developing the problem or else they would have been included in the program. If so, they may alter their behavior in risk enhancing ways. Because many not selected for intervention based on genotype will be at risk due to other risk-enhancing pathways, this could expose many to increased risk.

Finally, there is the problem of our increasing success in identifying relevant genotypes. Although we have focused primarily on two genotypes in our own research, it has become clear in the broader literature that there are a number of genetic markers that appear to be associated with susceptibility, vulnerability, or risk at various developmental stages and in different contexts (e.g. Belsky & Pluess, 2009). It is likely the list will continue to increase, and will become more complex as candidate gene approaches are increasingly replaced with gene-network approaches or other multi-locus approaches, resulting in longer lists of relevant genotypes. As the list of relevant genes and specific alleles becomes longer, the use of genotypes to exclude some individuals from participation in prevention programs will become increasingly difficult to justify. First, given a sufficient number of relevant genes in linkage disequilibrium, the number of “risk-free” individuals will become negligible. Likewise, multiple, independently distributed genes contributing to susceptibility should create an increasingly smooth curve (Gaussian) relating genotype to susceptibility or risk, confounding efforts to create clear “risk” and “non-risk” groups.

As may be clear already, we have paradoxically become less persuaded about the utility of genetic selection for intervention as the evidence of GxE effects based on well-studied copy number variants has increased. We continue to view genotyping as a useful tool for explicating alternative pathways to outcomes, exploring different biological mechanisms that may be more or less pertinent for particular individuals, and identifying and understanding boundary conditions for change. When it is necessary to move from universal prevention to indicated prevention, however, it may be more palatable to select on the basis of family history or phenotype rather than genotype. It seems unlikely that the current round of GxE results, or even a next round of more powerful, multi-gene index research, will provide an acceptable foundation for genetic screening for most preventive intervention, and the barriers seem particularly daunting for stigmatizing conditions and vulnerable populations.

Epigenetics and Family Science

As the literature on GxE effects involving families and GxI effects involving family intervention was expanding, another set of DNA related mechanisms took center stage among genetic researchers. The emergence of “epigenetic” measurement tools offered family and prevention science researchers another opportunity to identify biological links between family contexts and health or health behavior outcomes. Epigenetic mechanisms offer considerable promise for family researchers as they expand their etiological models. As was true for the addition of genetic polymorphisms to family-based research, there are a number of terminological and methodological considerations that are important. Accordingly, we begin with an admittedly simplified overview of terminology and basic concepts (See Philbert & Beach, 2015 for a more detailed overview). After we review terminology, we use our own recent research in this emerging area to highlight promising topics. In particular, we highlight the potential for epigenetics to address issues regarding the way that family and community context can get “under the skin” and so have relevance for problematic outcomes many years later. We also highlight the potential for epigenetics to help family researchers address theoretical issues regarding genetic vulnerability vs. susceptibility, family buffering effects, and the potential role of family in changing inflammatory processes.

Terminology and Basic Concepts

Why examine epigenetic modifications of DNA?—Epigenetic modifications refer to chemical additions to DNA that can be transmitted from a parent cell to a daughter cell, potentially changing gene regulation and expression, but not involving changes in the base pairs that make up the genetic code of DNA itself. That is, epigenetic modifications influence gene activity and expression without changing genotype. In some, but not all, cases these alternations also are transmitted to progeny, influencing outcomes for subsequent generations.

Changes in gene expression, i.e., the amount of mRNA produced by a gene, can influence outcomes of interest to family researchers. This is the same way that some genetic polymorphisms, such as the 5-HTTLPR, exert their effects. Accordingly, to the extent that epigenetic change can be linked to particular family or community contexts, it has the potential to explain, in part, the impact of those environments on gene expression, and provides a plausible biological mechanism by which both short and long-term changes in individual functioning could result from changing environments. For family researchers, changes in methylation (one type of epigenetic change) produced by environmental stressors, particularly early stressors, are of particular interest because alterations in methylation patterns may remain stable over a relatively long time in humans (Eckhardt, Lewin, Cortese, et al., 2006); consequently, individual differences in methylation may provide a potential biological marker of environmental contributions to the phenotypic divergence of those with similar genetic endowments (Fraga, Ballestar, Paz, et al., 2005), and a plausible physical substrate by which family processes may have lasting effects on health, health behavior, and other outcomes of interest. That is, epigenetic change may be

one mechanism by which family and community environments “get under the skin” to influence future outcomes.

Why focus on assessment of DNA methylation?—Currently, due to rapid advances in measurement technology, assessment of DNA methylation has become available to family researchers. There has been considerably more work done in model organisms and humans related to methylation than to other forms of epigenetic control, creating a substantial foundation of biologically plausible mechanisms for family scientists to explore. Early efforts to examine effects of DNA methylation were focused on particular gene regions or specific genes and gene networks, and as we note below, this may continue to be a useful strategy even as genomewide data sets become more widely available. For example, gene promoters and CpG islands, areas thought to be closely tied to gene regulation are likely to remain a focus of particular attention. The relationship between promoter DNA methylation and changes in gene activity are now relatively well established (Jaenisch & Bird, 2003; Murrell, et al., 2005). Hyper-methylation in promoter CpG islands typically results in decreased transcription of downstream genes (Stein, et al., 1982), whereas hypomethylation of promoter regions leads to an increase in gene transcription (e.g., Hansen & Gartler, 1990). This allows a priori predictions about the likely impact of changes in methylation. If the promoter for a gene is highly methylated one expects less gene expression on average. These characteristics make DNA methylation particularly attractive as a way to characterize biological change predictive of future stable intermediate behavioral phenotypes. Notwithstanding the current excitement regarding assessment of methylation patterns, it should be noted that an opposing process of “acetylation,” an epigenetic modification that typically results in greater gene expression, will also likely to be of considerable interest when measurement strategies become more tractable.

Does all methylation do the same thing?—A potential cautionary note is suggested by the fact that a large proportion of global DNA methylation occurs in non-coding regions, i.e., segments on the genome that do not code for protein sequences. Also, it is likely that DNA methylation evolved initially to protect organisms from genetic material left behind by viral intrusions rather than to fine tune regulatory processes, and methylation may continue to play this important role across the genome currently. In addition, because broad patterns of DNA methylation are established during early embryonic and fetal life and are essential to normal cellular development and differentiation, different cell-types have different characteristic methylation profiles (Uranov & Wolffe, 2001). The fact of cell-specific methylation patterns raises important measurement and methodological issues to which we shall return. In brief, methylation serves various functions and is not always serving the function of mediating environmental effects on phenotypic outcomes.

How does methylation work?—Methylation results in the addition of a methyl group into the major groove of DNA, potentially creating a physical barrier to transcription factor binding proteins and so inhibiting gene transcription at that site. At the same time, methylated DNA is more readily bound by proteins that initiate “chromatin remodeling”, a process that bends the sugar-phosphate backbone of DNA, ultimately generating a different shape for methylated DNA. In this way differential methylation of a region can lead to a

highly compact and entirely inactive structure, i.e., one that is tightly wound, permitting no interaction with the cellular environment and so no gene transcription, or a different conformation of the DNA backbone that is more open and permissive of gene activity. These and other changes induced by methylation alter gene expression, resulting in either an all or none change in expression or else a graduated effect on gene expression. More recent research has confirmed that DNA methylation patterns are predictive of individual differences in gene expression (e.g., Plume et al., 2012) and has confirmed the particular importance of methylation of promoter regions in predicting gene expression (Bell, Pai, Pickrell, 2011).

What are CpG Islands?—Of the base pairs comprising the DNA code, it is the CG pairs (also called CpG pairs), which are the primary targets of methylation. CG pairs are not randomly distributed across the human genome. In most regions of the human genome, the frequency of CpG dinucleotide pairs is much less than would be expected by chance. In those regions where CpG pairs do exist they are typically concentrated in areas known as “CpG islands.” In these areas there is a high density of CpG residues, and these CpG islands are often found in close proximity to gene promoters, first exons, or other regulatory elements, i.e., regions known to be important in regulating gene expression. Because dense arrays of “CpGs” frequently occur in areas affecting gene transcription, this makes the regions of DNA responsible for regulation of gene transcription more likely to be responsive to environmental input due to increased susceptibility to epigenetic regulation (i.e. greater susceptibility to methylation). This suggests that the genome may be prepared to respond to the environment and to modify various genetic programs based on experience.

Methodological issues in working with larger, genomewide data sets

Although methylation can be examined in single locations or in focused regions of interest, it has become increasingly common for it to be examined genomewide due to the availability of appropriate technologies. As data sets become larger, there is increasing need to perform a range of data cleaning and quality control steps to identify any errors and outliers. In addition, as a consequence of inevitable minor variations and minor procedural differences across “batches,” i.e., sets of data run through laboratory procedures at the same time, it is possible that “batch effects” will be introduced into the data set, reflecting procedural differences but not true biological differences. To correct for such effects it is important to “normalize” methylation array data and to then to correct for any remaining batch and chip effects as well as to take steps to control for individual differences due to variation in cell composition between participants. Below we briefly discuss these steps.

Quantile Normalization—When the Illumina 450K array (a common analytic platform for methylation assays) was developed, two different types of probes were included. The manufacturer recommended a relatively simple approach for determination of percent methylation at each CpG site without differentiation of the two different probe types. However, subsequent experience has shown that the two probe types have different distributional characteristics, and recent demonstrations (e.g. Pidsley et al., 2013) have shown that normalizing them separately produces superior results. Most authors now encourage separate normalization of Type I and Type II assays followed by use of quantile

normalization methods to improve detection of relationships with other variables by correcting distributional problems and also correcting minor batch variation problems.

Correcting Chip and Batch effects—Sun et al. (2011) have shown that quantile normalization as a first step in data processing typically reduces, but may not eliminate, batch and chip effects. Accordingly, after preprocessing to normalize the beta values within plates, all samples should be examined for batch and chip effects by using a box and density plot to indicate both the mean and confidence intervals around the mean in each case. Technical replicates across batches are also useful in confirming any suspected batch effect.

Controlling for mixed cell population—Unless the biological sample being used is comprised of a single cell type, an approach that has not been common practice among family scientists, there will also typically be a step in the analytic process that controls for variability in the distribution of cell types across individuals in the sample. Alternatively, in some cases, variation in the distribution of cell types may be a variable of interest in its own right. As Reinius and colleagues (2012), note, failure to control for effects of cell type can lead to potentially spurious associations if individual differences in predictors are also associated with individual differences in mixture of cell types. That is, researchers could attribute an effect to epigenetic change when it is better explained by shifts in the underlying cell populations comprising the sample.

Work on Epigenetic Effects in Specific Genes

Once methylation array data have been appropriately quality checked, cleaned and prepared for analysis they can be examined in a number of ways. Our initial work on DNA methylation focused on the influence of child abuse and other adverse environments on patterns of methylation in specific regions of candidate genes (Beach et al., 2010; 2011; 2013). Because of the strong signal environmental sent by extremely adverse environments, particularly those experienced in childhood, we hypothesized that child abuse would have a measurable impact on methylation. Child abuse has an effect on increased physiological reactivity (Heim & Nemeroff, 2001; Weiss, Longhurst, & Mazure, 1999), and so could plausibly lead to epigenetic change by inducing a substantial and sustained stress response; genomically, this was expected to result in change in methylation if areas relevant to transcription, i.e., methylation of promoters, 1st exons, or CpG islands. .

Focus on Methylation of 5HTT—We examined *5HTT* because of our previous focus on the genetic effects of this gene (see aforementioned studies in article) and because it is a highly conserved and phylogenetically old component of the serotonergic regulatory system, with a range of implications for behavioral dispositions that seemed to map on to some of the behavioral sequelae of child abuse (CDC, 2015). Likewise, primates exposed to a stressful environment during early development show altered central nervous system serotonin system functioning that produces long-term effects on behavior (Shannon et al., 2005). These effects include heightened aggressiveness, impaired impulse control (Ichise et al., 2006), and problematic social behavior (Mehlman et al., 1995).

In our initial investigation of the effects of child abuse on the methylation profiles at 5HTT, we found that abuse, comprised of physical abuse, harsh parenting, and sexual abuse were associated with overall hypermethylation of the 5HTT promoter region (Beach, Brody, Todorov, Gunter, Philibert, & 2010). A significant association also emerged in that sample between sexual abuse alone and overall methylation at 5HTT among female participants, $r(82) = .360, p < .001$. Thus, abuse (and sexual abuse specifically among women), appeared to have the potential to influence 5HTT transcription, potentially producing behavioral effects.

Is methylation of 5HTT a potential mediator?—In a follow-up, Beach et al. (2011) conducted an investigation of the impact of child abuse on changes in methylation in the promoter region of 5HTT (Beach et al., 2011), examining effects on antisocial and substance use tendencies. Given observations about the effect of serotonergic functioning on aggression, we proposed that hypermethylation of the promoter region of 5HTT might be one of the mechanisms by which the experience of child sex abuse contributes to increased risk for later antisocial behavior.

DNA for this study was prepared from immortalized lymphoblast cell lines derived from 155 female participants in the Iowa Adoptee Study. Methylation at 71 CpG residues in the promoter region of 5HTT was determined by quantitative mass spectroscopy and the resulting values were averaged to produce an average CpG ratio for each participant across the promoter region. Simple associations and path analyses within an Mplus framework were examined to characterize the relationships among childhood sex abuse by a family member, overall level of methylation, and subsequent antisocial behavior in adulthood. Because the sample was drawn from an adoption study, we were also able to control the effect of biological parent psychopathology as well as 5HTT genotype, and so control for additive gene effects.

Replicating the prior work (Beach et al., 2010), a significant effect of childhood sex abuse on methylation of the 5HTT promoter region emerged for women. In addition, a significant effect of methylation at 5HTT on symptoms of anti-social personality disorder (ASPD) emerged for women. This pattern of association suggested the possibility of mediation by epigenetic change. Using nested model comparisons, we supported the hypothesis that the direct effect of child sex abuse on symptoms of ASPD was fully mediated through methylation of 5HTT, suggesting that methylation of the promoter region of 5HTT in women is one potential biological mechanism linking child sex abuse to long-term changes in adult antisocial behavior in women. One important caveat is that comparable effects of child abuse on methylation of 5HTT were not observed in men in the replication sample. A second caveat is that the correspondence between methylation of lymphoblast cell lines and relevant brain regions has been established.

Does “Genetic Load” matter?—Completing this series of analyses (Beach et al., 2013), we next examined the impact of “genetic load” for psychopathology (i.e., having a biological parent with a diagnosed serious mental illness) on the observed association between childhood experiences of sex abuse (CSA) and methylation of the 5HTT promoter region among women. Because roughly half the women in the sample were known to have

parents with a psychiatric diagnosis whereas half did not, and all were adopted in the first year of life, they provided an opportunity to examine whether “genetic load” might amplify the impact of child abuse on methylation. We hypothesized that in addition to predicting adult psychopathology for women, genetic load would also moderate the impact of child sexual abuse (CSA).

Prior research with adoptees suggested that “genetic load” would moderate environmental effects. For example, Cadoret, Yates, Troughton, Woodworth, and Stewart (1995) found a main effect association between greater genetic load and increased likelihood of negative behavioral outcomes, as well as an interaction between genetic load and an adverse adoptive home environment. The interaction indicated that there was a negative effect of an adverse home environment for those youth who had greater genetic load, but not for those without genetic load. In keeping with this pattern, we anticipated that genetic load, in the form of having a biological parent with major psychopathology, would increase the deleterious impact of CSA on adult outcomes for women. In addition, we examined the possibility that genetic load would increase the impact of CSA on methylation.

In this analyses we expanded our definition of sex abuse to include all incidents of sex abuse before age 16, including those that did not involve family members. We also examined a wider range of adult outcomes, including depression and substance abuse. An advantage of the adoption design is that it minimizes the potential for passive gene-environment correlations (e.g., correlations of genetic load with family environment and adverse family events due to parent genetics and illness), providing additional support for the specific impact of sex abuse and associated adverse childhood events by ruling out potential genetic confounds and disentangling the impact of adverse childhood events from biological parent genetics.

Genetic load was considered positive if either biological parent had a history of substance abuse, antisocial behavior, or depression on the basis of agency, prison, hospital, or institutional records. Control adoptees without genetic load were matched to those positive for genetic load on the basis of age, sex, and age of mother at birth. Because CSA was reported 10 times more frequently by female than male participants in the broader sample, it was not practical to study the effects of CSA among males or to compare effects across gender. Accordingly, only the female portion of the sample ($N = 155$) was examined.

Two significant interaction effects involving CSA and “genetic load” were identified and plotted to examine the pattern of effects. In both cases the effect was in the hypothesized direction (i.e., there was greater impact of CSA for those with greater genetic load). The simple main effect of CSA on methylation among participants with greater genetic load was significant and strong ($p < .001$) whereas for those with no genetic load the effect was marginal ($p = .077$). Likewise, the simple main effect of CSA on symptoms of substance dependence among participants with greater genetic load was significant and strong ($p < .001$) whereas for those with no genetic load the effect was marginally significant in the same direction ($p = .055$).

Can we use methylation assessments to examine contrasting models of GxE effects?

Our initial series of analyses strongly suggested that, given a strong early environmental stimulus, it would be possible to identify changes in methylation, and that individuals with some genetic predispositions would show an intensified impact. However, this left open with the question as to whether any specific GxE effects would be discernable using more normative childhood stressors, such as adverse SES conditions during childhood, or specific genotypes thought to increase physiological reactivity to such conditions, such as the polymorphism at *5HTT*.

To address these questions we used a longitudinal sample of African American youth living in the Southern coastal plain, one of the most economically disadvantaged areas in the United States (Beach, Brody, Lei, Kim, Cui & Philibert, 2014). Because of our focus on those living in disadvantaged circumstances, substantial socio-economic status (SES) risk was experienced by a large percentage of the youth in the study, with potential effects on life expectancy (e.g., Braveman, Cubbin, Egerter, Willaims, & Pamuk, 2010), and other health effects across the lifespan (Starfield, Robertson, & Riley, 2002). We examined patterns of methylation across genes hypothesized to be linked to depressive symptoms, and related those patterns to more or less exposure to markers of socio-economic status (SES) risk as well as genetic variation at the 5-HTTLPR. We reasoned that using methylation as a dependent variable should provide a reliable and proximal window on patterns of GxE effects.

Comparing susceptibility and vulnerability models—We were particularly interested in using methylation data to contrast a genetic “vulnerability” model of 5-HTTLPR effects, in which “s” allele carriers are hypothesized to be less resilient to high levels of early stress, with a genetic “susceptibility” model in which they are hypothesized to respond more positively to low-stress, positive environments as well as more negatively to more difficult SES contexts (See Belsky & Pluess, 2009). To compare predictions from vulnerability and susceptibility models we used three non-redundant dependent variables created from the available methylation markers. First, we focused on a set of 50 CpG sites from depression related genes that were associated with the main effect of cumulative SES risk as well as with depressive symptoms in young adulthood. We chose this set of loci because it is neutral with regard to the presence or direction of any GxE effect, has the advantage of providing a direction of negative effects, clarifying interpretation, and avoids statistical concerns about identification of GxE effects in the absence of main effects. Second, we created an “interaction index” using all CpG sites from genes identified as depression-related that had nominally significant interaction terms. From this we constructed an index reflecting the total differential impact of cumulative SES risk for those with an “s” allele compared to those with only “l” or “vl” alleles. This index had the benefit of reflecting all reliable variance related to the interaction effect, but was neutral with regard to whether effects would reflect “vulnerability” or “susceptibility.” Finally, we examined all interaction effects on a genome-wide basis to test the hypothesis that “s” allele carriers would show increased impact of early cumulative SES risk at the genome-wide level.

African American families with a primary caregiver and a target youth from each of N = 388 families participated. Youth mean age was 11.7 years at the first assessment, 16 at the time

of genotyping based on saliva, and 19.2 years at the time of epigenetic assessment based on a blood draw. At both assessments, approximately half of the primary caregivers in the sample lived below federal poverty standards and could be characterized as working poor. With regard to genotype, we contrasted target youth who had one or two “s” alleles ($n = 152$, 39.18%) with all others ($n = 236$, 60.82%).

For the 50 “main effect” loci, we found a significant interaction with 5-HTTLPR genotype. The simple slopes conformed to expectations of greater impact of cumulative SES risk among “s” allele carriers, with a significant slope for “s” allele carriers ($p = .005$), but a non-significant slope among non-carriers ($p = ns$). This pattern resulted in a crossover effect of the sort predicted by the susceptibility model, i.e., the methylation index of “s” allele carriers was more sensitive to the effects of cumulative SES – in a for better or for worse manner compared to the non-carriers. Likewise, for our second dependent variable, the 300 CpG site “interaction index,” again we found a significant interaction of SES risk and genotype. “S” allele carriers showed a greater response to level of early, cumulative risk and, again, we found the cross-over pattern indicative of “susceptibility” effects. Finally, looking at all significant interaction effects across the genome, 23,864 out of a possible 25,601 times, the “s” allele carriers demonstrated greater absolute impact of cumulative risk on degree of methylation than their “l” or “vl” allele counterparts, deviating sharply from expectations under the null hypothesis.

In sum, using average epigenetic change across many genetic loci, Beach et al (2014) found an enhanced impact of early, cumulative SES risk among carriers of the *s* allele. Consistent patterns were observed across several complementary dependent variables. In each case, there was evidence of significantly increased impact of early, cumulative SES risk on methylation among *s* allele carriers relative to those with only *l* or *vl* alleles. These results provide an interesting initial illustration of the potentially useful role that methylation patterns may play in testing theoretical propositions, and prompted us to wonder if we also could use methylation patterns to test central propositions of prevention models positing a protective role for family processes. At the same time, we hoped that examination of methylation patterns might also allow us to probe the biological processes influenced when protective family processes counteract the negative effects of SES risk on long-term health outcomes. In particular we hoped to use methylation profiles to examine long-term effects on shifts in innate vs. acquired immune system functioning by examining individual differences in the proportion of different types of white blood cells circulating for participants.

Can we use methylation patterns to examine the impact of protective family processes on health?—Based on our earlier work we (Beach et al., in press) speculated that both SES-risk and protective parenting might have direct effects on health as well as having indirect effects via methylation of health-related genes. Because Illumina array methylation data can also be used to examine changes in the proportions of different mononuclear white blood cell types (e.g., Accomando, Weinke, Houseman, Nelson, Kelsey, 2014; Houseman et al, 2012), changes in cell type which may indicate shifts in pro-inflammatory patterns related to health, we were also interested in examining this potential pathway to health outcomes. Accordingly, using the longitudinal data from the SHAPE

sample (N = 398 families) described above we examined whether protective parenting and SES-risk might exert opposing effects on health by virtue of opposing impact on epigenetic patterns and/or cell-type variation. Protective parenting processes of support, communication, and monitoring and the adverse practice of harsh parenting (reverse coded) were assessed via target youth reports and parent reports during the first three waves of assessment (i.e. when youth were 11–13) and aggregated across multiple scales into a single index. SES related risk was assessed across the same time points, using the first three waves of parent reported SES related information. Youths' general health in young adulthood was based on self-reports obtained when they were 18 and 19 years old, i.e., shortly before and after the blood draw used to assess methylation. They reported their health using the five-item General Health Perceptions subscale from the RAND Short-Form Health Survey (Hays, Sherbourne, & Mazel, 1993). Methylation values were obtained using the Illumina (San Diego, CA) HumanMethylation450 Beadchip. All data were quality checked, quantile normalized, and corrected for batch effects. The resulting methylation data was used to characterize youth both with regard to health relevant loci and cell-type relevant loci.

Consistent with the broad hypotheses guiding the investigation, protective parenting and SES-risk were significantly negatively associated with each other and both were significantly associated with youth reports of health in young adulthood, suggesting that identification of shared mediators was possible. We first examined expectations that some loci would be associated with either SES-risk or protective parenting as well as being associated with youth-reported health. Using the 473,111 available loci in the cleaned methylation data set, excluding x and y chromosomes, and controlling for sex and age effects, there were 23,982 loci associated with parenting at $p < .01$, and 15 of these were significant genomewide at a False Discovery Rate (FDR) $< .05$ (Benjamini & Hochberg, 1995). This suggests the presence of substantial reliable signal linking parenting during childhood to methylation. SES-risk was also regressed on methylation genomewide, controlling for sex and age effects, identifying 28,640 loci that were associated at the $p < .01$ level of significance, and 2,032 loci associated at FDR $< .05$, again suggesting substantial reliable signal.

To identify potential shared biological pathways between parenting and SES-risk we looked for significant GoMiner™ categories in the set of genes linking youth reported health to parenting as well as to SES-risk. Ten categories of genes were identified as being significant at FDR $< .05$ for the loci common to protective parenting and health. For SES-risk and health, 144 categories of genes were identified as significant at FDR $< .05$. The three categories identified by GoMiner™ in both analyses were GO:0035466, regulation of signaling pathway; GO:0023033, signaling pathway, GO:0023052, signaling. These three categories indicated a potential shared biological mechanism of effect for both parenting and SES-risk on later young adult health. To create a methylation index that would capture this potentially shared mechanism we used all CpG sites associated with either protective parenting or SES-risk that were on the genes in the three common pathways.

We modeled hypothesized direct and indirect effects using Mplus. The outcome, youth reported health, was modeled as a continuous variable. We found that both parenting and SES risk exposure were associated significantly with the methylation index score as well as

with self-reported health in young adulthood. In addition, the impact of both protective parenting and SES-risk exposure on self-reported health in young adulthood was partially, but not fully, mediated by impact on the methylation index comprised of loci on the shared biological pathways. Unexpectedly, we found no significant mediation of parenting or SES-risk effects on health through individual differences in cell-type variation. However, various facets of cell type variation were significantly associated with the hypothesized mediator (the methylation index), with youth reported health, and with parenting, illustrating their value as control variables.

Our success in linking young adult health to earlier SES risk and parenting through broad categories of methylation, as well as linking parenting to shifts in the relative preponderance of particular, pro-inflammatory cell-types, caused us to wonder if it would be possible to use methylation data to examine more specific changes, possibly explicating the way that early experiences create a shift in preparedness for inflammatory responses later in life. If so, such shifts might also have implications as vulnerabilities for a number of longer-term health outcomes. This set the stage for our next set of analyses.

Can methylation of inflammatory genes identify vulnerabilities?—Given the apparent impact of family contexts on biologically relevant pathways for young adult health (Beach et al., in press), it was of particular interest to examine whether supportive family contexts during childhood might protect more specifically against the development of pro-inflammatory vulnerabilities. In keeping with predictive adaptive response models (PAR; Gluckman et al., 2005; Rickard and Lummaa, 2007) and related life course theory (Charnov, 1993; Cole et al., 2011), we predicted that negative family environment would influence later young adult inflammatory response tendencies (Beach et al., 2015), by upregulating the innate immune system. The innate immune system provides immediate defense against infection, and upregulation would be adaptive in response to an environment signaling elevated risk of physical threat (e.g. Irwin * Cole, 2011). A changed distribution of inflammation related cells tied to innate immunity, such as an increase in the proportion of monocytes (aka CD14 cells) relative to T or B cells (aka CD4, CD8, and CD19) could indicate a shift toward a pro-inflammatory response pattern. Conversely, protective parenting should reduce perceived threat of adversity for youth (Brody et al., 2004) and so decrease activity of the innate immunity system.

A second potential pathway of interest was through methylation of the first exon of *TNF*. Because the first exon is particularly predictive of gene expression (e.g., Plume et al., 2012), characterizing individual differences in methylation of inflammation-related genes in the region of the first exon may be particularly informative. We focused on *TNF* because it is the human gene that encodes *tumor necrosis factor* (TNF), often referred to as TNF α or TNF alpha. In humans this pro-inflammatory cytokine is commonly produced by activated macrophages, i.e. monocytes that have migrated to tissues and differentiated. Accordingly, as a central element of innate inflammatory response, epigenetic change via shifts in the level of methylation of *TNF* is a mechanism that may be important, and blood may be the correct tissue to examine to identify meaningful individual differences. Also, to the extent *TNF* methylation is established in response to important early developmental contexts, it may provide a window on latent vulnerability conferred by early experience. Focusing on

the eight CpG sites associated with the first exon of *TNF*, we hypothesized that greater methylation would result in reduced propensity for pro-inflammatory response and so better self-reported health.

Partially supporting our hypotheses regarding two mediational pathways, we found that protective parenting was associated significantly with the *TNF*-index ($p = 0.003$). However, our hypothesis regarding activation of the innate immune system via shifts in blood cell types fared less well. Protective parenting was associated significantly only with one of the three principle components comprising variation in cell types.,

To test the full model and examine competing pathways, we contrasted mediation from protective parenting to young adult health through the *TNF* index with mediation through the cell-type factor associated with protective parenting. The *TNF*-index was associated in the expected direction with cell-type variation, indicating that less methylation of *TNF* (pro-inflammatory) was associated with relatively greater presence of monocytes (pro-inflammatory). Using Mplus we obtained bootstrap confidence intervals for the effect of the independent variable (parenting) on the outcome variable (young adult self-reported health) through the mediator (TNF methylation index). We found the impact of protective parenting on self-reported health in young adulthood to be partially, but not fully, mediated by impact on the *TNF*-methylation index, accounting for 11.19% of the variance in young adult health. Cell-type variation, however, did not significantly mediate the effects of parenting on young adult health.

We concluded that protective parenting during late childhood and early adolescence influenced health in young adulthood, and potentially did so through associations with changes in methylation of the TNF promoter. To the best of our knowledge, this is the first study to contrast potential effects of protective parenting during late childhood and early adolescence on pro-inflammatory process through epigenetic regulation of gene expression vs. increased presence of pro-inflammatory innate immune system cell-types (e.g., monocytes). These findings are consistent with the proposition that poor health and health disparities during young adulthood may be ameliorated, in part, by processes correlated with protective parenting.

Conclusions and Future Directions

Our early work on GxE and GxI effects suggested that there was considerable individual variability in response to environments, including the manipulated environments produced by preventive intervention, and that this variability could be attributed to processes operating at the genetic level. This work suggested the need for better understanding of alternative, biologically based, pathways to disorder among youth growing up in challenging contexts that might expand family-based accounts of the development of disorder as well as resilience. The work was preliminary in that it focused on only a limited set of genes, and typically focused on only one gene at a time. The field is now entering a phase of attention to broader sets of genes and gene networks. It is likely that this new phase of research will substantially expand upon findings to date. At the same time, we concluded that use of gene indices for selection into prevention programs was not likely to become standard practice.

Rather, GxE research is more likely to continue as a basis for informing models of etiology and expanding the conceptual tools and etiological models available to design a new generation of preventive interventions.

One outgrowth of our work on GxE and GxI was an increased focus on the biological mechanisms that allow experiences in childhood to “get under the skin,” and then manifest later in adulthood as health risk and negative health outcomes. Because of the presence of chronic stressors associated with minority status, such as increased likelihood of exposure to poverty, neighborhood crime, and discrimination, understanding biological embedding may be particularly important for youth who are members of minority groups. These chronic stressors may lead some minority youth to experience accelerated weathering, and/or to develop persistent vulnerabilities to later health problems. Our research examining methylation indices suggests that methylation analyses provide a flexible tool that can be used to examine a range of potentially interesting questions related to development and the impact of family environments.

Early work on methylation of specific regulatory regions of candidate genes has provided considerable evidence that early environments can alter epigenetic signatures in various ways, potentially affecting health, health behavior, and psychopathology. More recent genomewide methylation approaches have addressed questions related to mechanisms of GxE effects. In addition, genomewide approaches have examined molecular mechanisms by which early experiences influence long-term health outcomes, and family-linked protective factors. There also appears to be growing potential to use methylation profiles to identify substance use exposure (e.g. Monick et al., 2012; Philibert et al., 2012), and as is discussed in the paper by Brody et al. (this volume) there is considerable potential to use methylation indices to identify processes involved in accelerated aging. Accordingly, the future seems likely to hold a range of interesting directions with considerable potential for productive intellectual exchange between family and prevention researchers on the one hand and epigenetic research on the other.

Because the area is still developing, there are many methodological issues yet to be fully addressed. We provided a very brief introduction to this growing literature along with references to primary sources for those interested in greater detail. Methylation provides an important tool for examining latent vulnerabilities that get “under the skin” to create future problems related to health and health behavior. As a result, there appears to be considerable important work ahead identifying the connections between the expanding tool kit provided by genetic researchers and the conceptual models that inform family science.

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