The emerging field of social and behavioral epigenetics

Connie J Mulligan

Abstract

Social and behavioral epigenetics is the study of psychosocial factors that impact biology through an epigenetic mechanism. Epigenetic modifications influence the activity of genes without altering the underlying DNA sequence. DNA methylation is one type of epigenetic modification that has been widely studied and found to associate with a broad range of psychosocial stressors. This paper reviews the landmark studies and current innovations. An evolutionary context for epigenetic changes induced by psychosocial stress, and the possible heritability of such changes, is also presented. The involvement of social and behavioral scientists in this emerging field is essential to ensure that the nuances of the psychosocial environment are well understood and accurately modeled.

INTRODUCTION

Social and behavioral epigenetics examines the role of epigenetic modifications to mediate the effect of psychosocial stressors on an individual. Researchers in this emerging field investigate a range of outcomes such as an individual's health, cognition and behavior. Negative psychosocial factors, like early life adversity, are thought to play a particularly important role in an individual's lifelong health and well-being. The impact of prenatal stressors, such as undernutrition, on adult health led to the Developmental Origins of Health and Disease (DOHaD) hypothesis, first proposed by Barker over 30 years ago (Barker, 2007; Barker & Osmond, 1986). Social and behavioral epigenetics builds on the DOHaD framework by adding psychosocial stressors to the list of impactful early life stressors and by explicitly proposing an epigenetic mechanism to translate lived experiences into altered biological conditions.

Epigenetic modifications directly impact biology by altering the activity of genes, which can lead to changes in the condition, or **phenotype**, of an individual. Genes are vital parts of the genome that produce the functional molecules, typically proteins, that create an individual's unique phenotype. Epigenetic modifications do not change the underlying DNA sequence of the genome, but are one of the ways in which the activity, or expression, of genes can be influenced. **DNA methylation** is an important type of epigenetic modification wherein **methyl groups** are added to the existing DNA sequence, most often at a **cytosine** followed by a **guanosine**, i.e. a CpG site. Originally, DNA methylation was found to 'silence' genes, or turn off their expression, when methylation occurred in the **promoter** region before the start of a gene. More recently, research has shown that gene expression can be either increased or decreased depending on the region of the gene that is methylated as well as the cell and tissue type (Jones, 2012; Plongthongkum, Diep, & Zhang, 2014).

Epigenetics has been studied for decades by molecular biologists who focus on molecular mechanisms, such as how methylation at one particular site affects the expression of a gene. More recently, social scientists have started to participate in epigenetic research in order to provide an essential perspective on human health and well-being that includes the social, psychological, and behavioral dimensions (e.g. Hall, 2014).

Using a social and behavioral epigenetics framework, the prediction is that lifetime social and behavioral stressors produce changes in DNA methylation that lead to changes in gene expression that lead to changes in condition or phenotype. Furthermore, the altered condition may then feedback to influence the process in a cyclical manner. For example, poverty may create certain epigenetic changes that alter the expression of genes that increase an individual's risk of developing depression that then further entrenches the individual in poverty. The full range of psychosocial factors that individuals experience, including both negative and positive events, may leave epigenetic marks that continue to

affect individuals throughout their lives. Thus, social scientists bring a unique perspective that is essential to fully understanding the complexities of health and well-being throughout the life course.

Even though epigenetic modifications do not alter the underlying DNA sequence, it is possible that certain epigenetic modifications may be heritable. The heritability of psychosocial stressor-induced epigenetic marks creates the possibility that individuals' responses to social and behavioral stressors experienced during their lifetime may be passed on to future generations. Transgenerational inheritance of psychosocial stressor-induced epigenetic changes is one of the most controversial aspects of social and behavioral epigenetics. Even if only a small set of genes is subject to heritable, psychosocial stressor-induced epigenetic modification, that intriguing possibility suggests that both Darwin and Lamarck might have been correct in aspects of their theories of evolution and heritability. Furthermore, the possibility of a heritable epigenetic signature of psychosocial stress has profound implications for our understanding and attempts to ameliorate some of society's most vexing problems, including multigenerational cycles of violence, abuse and poverty.

FOUNDATIONAL RESEARCH

Over the past decade, the field of social and behavioral epigenetics has continued to emerge and knowledge gaps have been identified as multiple disciplines contribute to the effort, creating a truly transdisciplinary field. The search for epigenetic signatures of social and behavioral factors began in the early 2000s. In 2004, Szyf and Meaney published the most highly cited paper ever in *Nature Neuroscience* (over 3000 citations) entitled 'Epigenetic programming by maternal behavior' (Weaver *et al.*, 2004). They identified differences in DNA methylation in the brains of rat offspring that associated with differences in maternal nurturing behaviors, i.e. pup licking and grooming, arched-back nursing. The changes in DNA methylation occurred at the **glucocorticoid receptor gene** (short name = GR), which is a gene involved in the **HPA axis** response to stress. The methylation changes affected the ability of the GR gene to produce its protein. These differences only emerged after the first week of life when the behavioral differences between high and low nurturing mothers were also most apparent. And the DNA methylation patterns persisted into adulthood demonstrating a possible mechanism for the long-lasting effect of early psychosocial events. Furthermore, the methylation differences were reversible with cross-fostering of the rat pups, i.e. within 12 hours of birth, if biological offspring of high and low nurturing mothers were cross-fostered to low and high nurturing mothers, respectively, they developed the methylation profile associated with the rearing mother. These results suggest that the DNA methylation differences in the offspring were not merely correlational, but were a direct response to maternal nurturing behavior.

Five years later, Meaney and Szyf demonstrated similar changes in DNA methylation in humans. Specifically, they identified significant differences in DNA methylation in the human version of the glucocorticoid receptor gene (short name = *NR3C1*) in suicide victims with a history of childhood abuse relative to suicide victims with no history of childhood abuse and non-suicide controls (McGowan *et al.*, 2009). Furthermore, they found increased DNA methylation and decreased *NR3C1* expression in the abused suicide victims that is consistent with the known effect of DNA methylation in gene promoters on gene expression. These results suggest that the early childhood abuse and later suicide may have been causal and mediated by the methylation and expression changes in the *NR3C1* gene.

Since these ground-breaking studies, many more papers have been published that report changes in DNA methylation associated with a diverse range of psychosocial stressors. For instance, multiple studies have shown an epigenetic effect of socioeconomic status (SES), with childhood status impacting adult methylation more than adult status (Borghol *et al.*, 2012; McDade *et al.*, 2017a; Needham *et al.*, 2015). Also, Fumagalli *et al.* (2018) found associations between early life stress (i.e. very preterm birth), DNA methylation at the serotonin transporter gene (this gene, *SLC6A4,* is involved in a range of conditions including PTSD and depression-susceptibility in trauma-exposed individuals), and socio-

emotional development at 12 months, demonstrating a role for DNA methylation in the influence of psychosocial stressors after the initial exposure. A number of studies have shown that prenatal exposure to maternal stress is associated with changes in DNA methylation in offspring and altered health outcomes such as birthweight, infant cortisol stress response, and expression of genes involved in immune functions (Mulligan, D'Errico, Stees, & Hughes, 2012; Nemoda & Szyf, 2017; Oberlander *et al.*, 2008).

Some epigenetic studies have focused on more controversial topics, such as the biological basis of sexual orientation. Using a mouse model, Vilain's group found that perinatal exposure to testosterone induced relatively modest methylation changes in the brain at birth but that 20-fold more genes exhibited differential methylation in the adult (Ghahramani *et al.*, 2014). This impact of early hormone exposure on adult methylation was independent of adult hormone levels. Vilain's group also studied masculinized women to test these results in humans. Specifically, women who were exposed to high levels of testosterone *in utero* due to a genetic condition that produces excessive testosterone (called congenital adrenal hyperplasia) showed much higher rates of non-heterosexual orientation than non-exposed women and Ngun and Vilain (2014) suggest an epigenetic mechanism to mediate the long-term effects of hormone exposure.

GROWING PAINS OF AN EMERGING FIELD

Given the exponential growth in the number of published studies, there is concern that the role for epigenetics has been overstated, particularly with respect to the influence of social and behavioral factors on DNA methylation (Miller, 2010). In response, some researchers have developed hypotheses to test in humans based on results from animal models and they have come up empty-handed. For instance, University of British Columbia researchers hypothesized that SES might be analogous to the nurturing behaviors in rats in Szyf and Meaney's studies (see section above) and they predicted increased methylation at *NR3C1* in association with low SES. However, they did not find any evidence of altered DNA methylation despite seeing the expected reduced glucocorticoid response and increased cortisol indicative of a stress response (Chen, Miller, Kobor, & Cole, 2010; Miller *et al.*, 2009). Other groups (Rijlaarsdam *et al.*, 2016; Ryan, Mansell, Fransquet, & Saffery, 2017) have used meta-analyses to look for common results across multiple studies or tested new population samples to confirm previous results and have found no association of maternal stress and newborn DNA methylation in contrast to published studies, including those listed in the previous section. However, when combining studies in meta-analyses, it almost always means that different stress measures are combined, i.e. depression, anxiety, intimate partner violence, etc, or that new composite stress measures are created, so that the meta-analysis is testing a different hypothesis than the original studies. This issue is particularly salient when moving from animal models to humans, e.g. SES may not be the most appropriate human analog for nurturing behaviors in rat mothers. Furthermore, when attempting to replicate results in new populations, it is possible that the original results are valid but do not manifest in the same way in other populations. This lack of replication may be especially likely with DNA methylation studies where we are still learning exactly how DNA methylation impacts gene expression and phenotype, e.g. it is possible that the same stress exposure may alter methylation at different CpG sites in different populations but may have similar functional effects on gene expression and outcome.

An instructive set of comments and responses were published by Szyf's and Kobor's groups in response to the previously mentioned study of SES and DNA methylation by Borghol *et al.* (2012). Lam et al. (2012) questioned whether the statistical approach used by Borghol *et al.* (2012) to analyze CpG sites was appropriate and questioned why Borghol *et al.* (2012) found so many CpG sites associated with SES (n=1252) when Lam *et al.* (2012) found only three associated sites. In their response, Suderman & Borghol *et al.* (2013) point out that both studies found associations of early life SES and DNA methylation despite differences in methods (the DNA methylation datasets were generated using

different platforms) and different populations (US vs UK). They further point out that their intent was not to claim that particular CpG sites were specifically modified by early life SES, but to establish that early life SES was generally associated with DNA methylation in adult blood samples, a result found by both groups and captured in their title – "Epigenomic socioeconomic studies more similar than different". In their second response, Lam et al. (2013) focus on the possibility that Borghol *et al.* (2012) did not properly account for differences in types of cells in whole blood samples and they propose that cell type differences could be driving the association with SES rather than methylation differences. Ultimately, however, they conclude that "both of our studies support a general association of early-life SES and adult DNA methylation" (Lam *et al.*, 2013: E1247). This is an enlightening exchange because it illustrates, publicly and in some detail, how different scientists can interpret the same results in different ways depending on their perspective and expectations, i.e. was the critical result the identification, and replication, of specific CpG sites or a more general association between DNA methylation and SES?

The Szyf and Kobor papers also highlight the problem of cell type heterogeneity, which has emerged as an important issue in epigenetic studies. Venous blood is composed of multiple cell types, including erythrocytes, leucocytes, and platelets, and the proportions of these cells can change in response to stress. Furthermore, each cell type has unique epigenetic marks so a change in cell type could result in an altered epigenetic signal even though the epigenetic change was not a direct result of the stress exposure. The solution is to control for cell type heterogeneity so that only epigenetic changes above and beyond those associated with changes in cell types are measured. Multiple methods papers have now been published to allow correction for cell type heterogeneity in different tissues (e.g. Houseman *et al.*, 2012). In our study of prenatal exposure to maternal stress in mother-newborn dyads in the Democratic Republic of Congo (DRC), we originally found associations between maternal stress and DNA methylation in both maternal venous blood and newborn cord blood samples, but after correction for cell type differences, only the associations in maternal blood remained (Clukay, Hughes, Rodney, Kertes, & Mulligan, 2018).

Good science is self-correcting. Several issues have emerged in epigenetic studies that are actively being investigated and addressed. Correction for cell type heterogeneity is one such issue - solutions to the problem continue to be developed and their use is becoming standard in current studies. Another active area of study is the ability of easily accessible tissues, like blood and saliva, to accurately reflect stress responses that primarily occur in the brain or other tissues. Most studies addressing this issue have compared DNA methylation changes in multiple tissues and they usually find different methylation profiles between different tissue types (Agha *et al.*, 2015; Hannon, Lunnon, Schalkwyk, & Mill, 2015). However, epigenetically-determined changes in gene expression are part of the differentiation process by which cells with the same genome become different types of cells. Thus, different methylation profiles in different tissues are expected. The question remains, are there methylation differences, above and beyond the tissue-specific differences, that associate with a stressor or outcome of interest and that are congruent across tissues? Few studies have directly addressed this question.

PUTTING THINGS IN PERSPECTIVE

In order to step back and address the social and behavioral epigenetic skeptics, it is useful to think about the questions that define the field. Most fundamentally, does it make biological or evolutionary sense that DNA methylation could be sensitive to psychosocial stressors? If so, what would the methylation signatures look like and where in the genome would we look for them? Would different stressors leave different signatures? How long might these methylation signatures persist? A few months? Years? Generations? Could methylation signatures at different genes persist for different periods of time?

An evolutionary perspective is useful when pondering these questions. Epigenetically-influenced changes in gene expression in response to psychosocial stress may have evolved in order to provide rapid, short-term responses to changes in the psychosocial environment without changing the

underlying DNA sequence (Mulligan, 2016). In contrast, genetic changes to the genome sequence would provide long-term adaptation to the environment since they occur more infrequently over many generations. Epigenetic response to psychosocial stressors may have evolved in humans as an adaptation to increasingly complex stressors that are not experienced by simpler organisms; for example, contrast the experience of sexual violence in humans to the heat exposure that is used to elicit a stress response in bacteria. Furthermore, some environmentally-sensitive epigenetic signatures may have evolved to be transmitted and maintained in future generations so as to preserve information about the original stressor; these would be heritable, environmentally-induced epigenetic modifications.

The number of genes involved in an epigenetic response to a psychosocial stressor is likely to be small relative to the ~20,000 genes in the human genome since the majority of genes must continue to function regardless of changes in the environment. These epigenetically-modifiable genes may have evolved to be sensitive to environmental cues in order to improve adaptability and fitness. In our study of prenatal exposure to maternal stress in the DRC, we found that only 212 CpG sites, out of >400,000 studied sites, correlated with maternal stress (with a false discovery rate of 5%), suggesting a very small number of environmentally-sensitive, modifiable CpG sites (Rodney & Mulligan, 2014).

Furthermore, it is possible that only extreme stressors will leave strong and easily detectable epigenetic marks on the genome, on the assumption that humans and other organisms have evolved to tolerate every-day stressors. That is not to say that more moderate stressors do not leave an epigenetic signature, but that such an epigenetic signature may be weaker or more diffuse across the genome and, therefore, more difficult to detect. In support of the idea that extreme stressors have the biggest impact, we found that war stress and personal experience of rape had the greatest effect on newborn DNA methylation and birthweight when compared to milder stressors like material deprivation and mundane stress (Mulligan *et al.*, 2012). Study of extreme stressors may help inform studies of more moderate stressors by identifying the genes, gene contexts (e.g. promoters vs enhancers), and parts of the genome with the most environmentally-sensitive, epigenetically-modifiable sites, thus allowing future studies to focus on those sites.

APPLICATIONS AND FUTURE DIRECTIONS

The implications that an individual's experiences can leave a permanent mark, all the way down to the genome, that may persist throughout an individual's lifetime are enormous and thought-provoking. An obvious question is, can this information be used to help people? The fact that methylation marks are changeable suggests that we may be able to intervene in cases of early life adversity to improve later life health and well-being.

Currently, multiple studies are searching for 'epi-signatures' of particular conditions that will allow more accurate diagnosis of disease and earlier identification of conditions that would benefit from early intervention. Aref-Eshghi *et al.* (2018) identified DNA methylation signatures in venous blood samples that were specific for nine out of 14 tested neurodevelopmental syndromes, thus allowing for more accurate diagnosis and early treatment. In a study of alcohol dependence, Brückmann *et al.* (2016) found that hypomethylation of the *GDAP1* gene (a member of the ganglioside-induced differentiation-associated protein family that is involved in neuronal development) was a biomarker for disease severity. Furthermore, the hypomethylation was reversed during an alcohol treatment program, suggesting that *GDAP1* methylation could also be used as a biomarker for treatment outcome and highlighting the lability of DNA methylation marks.

Studies of epi-signatures of psychosocial stressors with a predictive application for future conditions are more limited. McDade *et al.* (2017b) identified psychosocial and biological exposures that predicted DNA methylation at genes involved in inflammation, which is a risk factor for multiple diseases of aging. In a study of childhood stress, Natt *et al.* (2015) found changes in DNA methylation in 5-year-olds similar

to those seen in normal aging, suggesting that these DNA methylation changes may help predict future disease susceptibility.

The studies listed above suggest promise for the use of DNA methylation as a biomarker for different stress exposures and resultant health outcomes. But what about purposely manipulating DNA methylation to improve health and well-being? Some intriguing studies have been conducted in animal models. Dietary supplementation of genistein to pregnant mice caused a striking shift in coat color in their offspring and was associated with increased methylation upstream of the pigment-producing *Agouti* gene (Dolinoy, Weidman, Waterland, & Jirtle, 2006). Furthermore, the genistein-induced hypermethylation persisted into adulthood and protected offspring from obesity. Genistein is a plant-derived estrogen, found in soy, that has been linked to cancer prevention and the levels of *in utero* supplementation used in the study were comparable to levels in humans who consume high-soy diets. In another study, researchers tested the idea that DNA methylation may directly influence social behavior by manipulating DNA methylation in order to alter social status in African cichlid fish (Lenkov, Lee, Lenkov, Swafford, & Fernald, 2016). Low-status animals who were injected with DNA methylating agents were statistically likely to increase in social rank whereas those injected with demethylating agents were statistically unlikely to increase in rank.

If the animal model results translate to humans, they suggest we may be able to devise treatments to reverse epigenetic alterations made in response to stress exposures, albeit with a lot of additional study. In our DRC study, we hope to study breastfeeding as a treatment to mitigate the effects of prenatal exposure to maternal stress and test if the DNA methylation profile changes from a high-stress profile to a low-stress one. Ultimately, successful intervention will depend on robust measurement and modeling of the nuances of the psychosocial environment as well as a detailed understanding of the epigenetic mechanisms that mediate the impact of psychosocial stressors on health and well-being.

The large number of published studies may give a false impression that we have a firm understanding of the epigenetic impact of stressors and, furthermore, that every kind of stressor leaves an epigenetic signature. However, as in any emerging field, initial reports tended to focus on positive results. Recently the field has begun to mature to the point that negative associations are being published, e.g. no association found between victimization during childhood and DNA methylation (Marzi *et al.*, 2018). Publication of negative results is a good step forward since, from an evolutionary perspective, it does not make sense that every stressor we experience will alter our DNA methylation and gene expression and subsequent phenotypes. It is not yet possible to predict which stressors will leave an epigenetic mark, and which ones will not, so we must study the effect of a wide range of stressors and publish both positive and negative findings.

SUMMARY

- Social and behavioral epigenetics is the study of psychosocial factors that impact biology through a proposed epigenetic mechanism.
- Epigenetic responses to psychosocial stressors may have evolved in order to provide rapid, short-term responses to changes in the psychosocial environment without changing the underlying DNA sequence.
- Some environmentally-induced epigenetic changes may be heritable in order to preserve information across generations about past stressful exposures.
- Many studies have found associations between DNA methylation and a wide range of psychosocial stressors, including direct and prenatal exposures.
- The number of genes, and regions of the genome, that are sensitive to the psychosocial environment and epigenetically-modifiable is likely to be small.

- Future studies should investigate a wide range of psychosocial stressors, in multiple populations at different ages and stages of development, by assaying an increasingly complete set of epigenetic modifications across multiple genes and regions of the genome.
- The publication of positive and negative results is critical in order to better understand which stressors are processed through an epigenetic mechanism.

GLOSSARY

Methyl group – one carbon molecule plus three hydrogen molecules
DNA methylation – attachment of a methyl group at a specific position in the DNA sequence
Cytosine, guanosine – two of the four variable parts of a DNA sequence, i.e. cytosines, guanosines, adenosines and thymines
Promoter – the region before the start of a gene that helps control how much protein is made from the gene
Phenotype - the observable characteristics of an organism, including morphology, development, physiology and behavior
Glucocorticoid receptor gene – the gene that encodes the receptor that binds glucocorticoid hormones, such as cortisol, and is involved in the the HPA-axis stress response
HPA axis – the hypothalamo-pituitary-adrenal axis of organs and hormones that mediates the body's automatic response to stress.

ACKNOWLEDGMENTS

Sincere gratitude is due to the study participants from Jordan and Syria, the Democratic Republic of Congo and Tallahassee, FL, without whom none of my research would be possible. Appreciation goes to current and past Mulligan lab members. Funding was provided by NSF grants BCS 1719866, 1448213, 1231264 and 0820687 and grants from the University of Florida (UF) Clinical and Translational Science Institute, UF College of Liberal Arts and Science, and a UF Research Opportunity Seed Fund award.

References

Agha, G., Houseman, E. A., Kelsey, K. T., Eaton, C. B., Buka, S. L., & Loucks, E. B. (2015). Adiposity is associated with DNA methylation profile in adipose tissue. *International Journal of Epidemiology, 44*(4), 1277-1287. doi:10.1093/ije/dyu236

Aref-Eshghi, E., Rodenhiser, D. I., Schenkel, L. C., Lin, H., Skinner, C., Ainsworth, P., . . . Sadikovic, B. (2018). Genomic DNA Methylation Signatures Enable Concurrent Diagnosis and Clinical Genetic Variant Classification in Neurodevelopmental Syndromes. *The American Journal of Human Genetics, 102*(1), 156-174. doi:10.1016/j.ajhg.2017.12.008

Barker, D. J. (2007). The origins of the developmental origins theory. *J Intern Med, 261*(5), 412-417. doi:10.1111/j.1365-2796.2007.01809.x

Barker, D. J., & Osmond, C. (1986). Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet, 1*(8489), 1077-1081.

Borghol, N., Suderman, M., McArdle, W., Racine, A., Hallett, M., Pembrey, M., . . . Szyf, M. (2012). Associations with early-life socio-economic position in adult DNA methylation. *International Journal of Epidemiology, 41*(1), 62-74. doi:10.1093/ije/dyr147

Brückmann, C., Di Santo, A., Karle, K. N., Batra, A., & Nieratschker, V. (2016). Validation of differential GDAP1 DNA methylation in alcohol dependence and its potential function as a biomarker for disease severity and therapy outcome. *Epigenetics, 11*(6), 456-463. doi:10.1080/15592294.2016.1179411

Chen, E., Miller, G. E., Kobor, M. S., & Cole, S. W. (2010). Maternal warmth buffers the effects of low early-life socioeconomic status on pro-inflammatory signaling in adulthood. *Molecular Psychiatry, 16*, 729. doi:10.1038/mp.2010.53

Clukay, C. J., Hughes, D. A., Rodney, N. C., Kertes, D. A., & Mulligan, C. J. (2018). DNA methylation of methylation complex genes in relation to stress and genome-wide methylation in mother–newborn dyads. *Am J Phys Anthropol, 165*(1), 173-182. doi:10.1002/ajpa.23341

Dolinoy, D. C., Weidman, J. R., Waterland, R. A., & Jirtle, R. L. (2006). Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. *Environ Health Perspect, 114*(4), 567-572.

Fumagalli, M., Provenzi, L., De Carli, P., Dessimone, F., Sirgiovanni, I., Giorda, R., . . . Montirosso, R. (2018). From early stress to 12-month development in very preterm infants: Preliminary findings on epigenetic mechanisms and brain growth. *PLoS ONE, 13*(1), e0190602. doi:10.1371/journal.pone.0190602

Ghahramani, N. M., Ngun, T. C., Chen, P.-Y., Tian, Y., Krishnan, S., Muir, S., . . . Vilain, E. (2014). The effects of perinatal testosterone exposure on the DNA methylome of the mouse brain are late-emerging. *Biology of Sex Differences, 5*(1), 8. doi:10.1186/2042-6410-5-8

Hall, S. S. (2014). Behaviour and biology: The accidental epigeneticist. *Nature, 505*, 14-17.

Hannon, E., Lunnon, K., Schalkwyk, L., & Mill, J. (2015). Interindividual methylomic variation across blood, cortex, and cerebellum: implications for epigenetic studies of neurological and neuropsychiatric phenotypes. *Epigenetics*, 00-00. doi:10.1080/15592294.2015.1100786

Houseman, E. A., Accomando, W. P., Koestler, D. C., Christensen, B. C., Marsit, C. J., Nelson, H. H., . . . Kelsey, K. T. (2012). DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics, 13*(1), 86. doi:10.1186/1471-2105-13-86

Jones, P. A. (2012). Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet, 13*(7), 484-492.

Lam, L. L., Emberly, E., Fraser, H. B., Neumann, S. M., Chen, E., Miller, G. E., & Kobor, M. S. (2012). Factors underlying variable DNA methylation in a human community cohort. *Proceedings of the National Academy of Sciences, 109*(Supplement 2), 17253-17260. doi:10.1073/pnas.1121249109

Lam, L. L., Emberly, E., Fraser, H. B., Neumann, S. M., Chen, E., Miller, G. E., & Kobor, M. S. (2013). Reply to Suderman et al.: Importance of accounting for blood cell composition in epigenetic studies. *Proceedings of the National Academy of Sciences, 110*(14), E1247-E1247. doi:10.1073/pnas.1222104110

Lenkov, K., Lee, M. H., Lenkov, O. D., Swafford, A., & Fernald, R. D. (2016). Epigenetic DNA Methylation Linked to Social Dominance. *PLoS One, 10*(12), e0144750. doi:10.1371/journal.pone.0144750

Marzi, S. J., Sugden, K., Arseneault, L., Belsky, D. W., Burrage, J., Corcoran, . . . Caspi, A. (2018). Analysis of DNA Methylation in Young People: Limited Evidence for an Association Between Victimization Stress and Epigenetic Variation in Blood. *American Journal of Psychiatry, 0*(0), appi.ajp.2017.17060693. doi:10.1176/appi.ajp.2017.17060693

McDade, T. W., Ryan, C., Jones, M. J., MacIsaac, J. L., Morin, A. M., Meyer, J. M., . . . Kuzawa, C. W. (2017a). Social and physical environments early in development predict DNA methylation of inflammatory genes in young adulthood. *Proc Natl Acad Sci U S A, 114*(29), 7611-7616. doi:10.1073/pnas.1620661114

McDade, T. W., Ryan, C., Jones, M. J., MacIsaac, J. L., Morin, A. M., Meyer, J. M., . . . Kuzawa, C. W. (2017b). Social and physical environments early in development predict DNA methylation of inflammatory genes in young adulthood. *Proceedings of the National Academy of Sciences*. doi:10.1073/pnas.1620661114

McGowan, P. O., Sasaki, A., D'Alessio, A. C., Dymov, S., Labonte, B., Szyf, M., . . . Meaney, M. J. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci, 12*(3), 342-348.

Miller, G. (2010). Epigenetics. The seductive allure of behavioral epigenetics. *Science, 329*(5987), 24-27.

Miller, G. E., Chen, E., Fok, A. K., Walker, H., Lim, A., Nicholls, E. F., . . . Kobor, M. S. (2009). Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. *Proceedings of the National Academy of Sciences, 106*(34), 14716-14721. doi:10.1073/pnas.0902971106

Mulligan, C. J. (2016). Early environments, stress, and the epigenetics of human health. *Annual Review of Anthropology, 45*, 233-249.

Mulligan, C. J., D'Errico, N. C., Stees, J., & Hughes, D. A. (2012). Methylation changes at NR3C1 in newborns associate with maternal prenatal stress exposure and newborn birth weight. *Epigenetics, 7*(8), 853-857. doi:10.4161/epi.21180

Nätt, D., Johansson, I., Faresjö, T., Ludvigsson, J., & Thorsell, A. (2015). High cortisol in 5-year-old children causes loss of DNA methylation in SINE retrotransposons: a possible role for ZNF263 in stress-related diseases. *Clinical Epigenetics, 7*(1), 91. doi:10.1186/s13148-015-0123-z

Needham, B. L., Smith, J. A., Zhao, W., Wang, X., Mukherjee, B., Kardia, S. L. R., . . . Diez Roux, A. V. (2015). Life Course Socioeconomic Status and DNA Methylation in Genes Related to Stress Reactivity and Inflammation: The Multi-Ethnic Study of Atherosclerosis. *Epigenetics*, 00-00. doi:10.1080/15592294.2015.1085139

Nemoda, Z., & Szyf, M. (2017). Epigenetic Alterations and Prenatal Maternal Depression. *Birth Defects Research, 109*(12), 888-897. doi:10.1002/bdr2.1081

Ngun, T. C., & Vilain, E. (2014). Chapter Eight - The Biological Basis of Human Sexual Orientation: Is There a Role for Epigenetics? In D. Yamamoto (Ed.), *Advances in Genetics* (Vol. 86, pp. 167-184): Academic Press.

Oberlander, T. F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., & Devlin, A. M. (2008). Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics, 3*(2), 97-106.

Plongthongkum, N., Diep, D. H., & Zhang, K. (2014). Advances in the profiling of DNA modifications: cytosine methylation and beyond. *Nat Rev Genet, 15*(10), 647-661. doi:10.1038/nrg3772

Rijlaarsdam, J., Pappa, I., Walton, E., Bakermans-Kranenburg, M. J., Mileva-Seitz, V. R., Rippe, R. C. A., . . . van Ijzendoorn, M. H. (2016). An epigenome-wide association meta-analysis of prenatal maternal stress in neonates: A model approach for replication. *Epigenetics*, 1-10. doi:10.1080/15592294.2016.1145329

Rodney, N. C., & Mulligan, C. J. (2014). A biocultural study of the effects of maternal stress on mother and newborn health in the Democratic Republic of Congo. *Am J Phys Anthropol, 155*(2), 200-209. doi:10.1002/ajpa.22568

Ryan, J., Mansell, T., Fransquet, P., & Saffery, R. (2017). Does maternal mental well-being in pregnancy impact the early human epigenome? *Epigenomics*. doi:10.2217/epi-2016-0118

Suderman, M., Borghol, N., Pappas, J. J., McArdle, W., Racine, A., Hallett, M. T., . . . Szyf, M. (2013). Epigenomic socioeconomic studies more similar than different. *Proceedings of the National Academy of Sciences, 110*(14), E1246. doi:10.1073/pnas.1221019110

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., . . . Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nat Neurosci, 7*(8), 847-854.

CONNIE J MULLIGAN SHORT BIOGRAPHY

Connie J Mulligan is a professor in the Department of Anthropology and the Genetics Institute at the University of Florida, Gainesville, Florida. She conducts research on epigenetic and genetic signatures of psychosocial stress. Her projects include the investigation of an epigenetic mechanism to mediate the impact of maternal stress on newborns in the Democratic Republic of Congo, the genetics and epigenetics of exposure to war trauma in Syrian refugees, and the genetic and biocultural risk factors for complex diseases and related racial disparities in African Americans living in Tallahassee. She was Chair of the Academic Organizing Committee of the NSF/NIH/RCUK Interagency Epigenetics Workshop Advisory Board and, in 2014, she organized an international workshop on social and behavioral epigenetics that included both social and biological scientists (see workshop report at www.nichd.nih.gov/about/meetings/2014/Documents/ExecSocialBehavEpigenetics_Sum.pdf). Mulligan is the current President of the American Association of Anthropological Genetics. For more information, see http://users.clas.ufl.edu/cmulligan/Webpage/.