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Principles and Challenges of Applying Epigenetic Epidemiology to Psychology

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Abstract

The interplay of genetically driven biological processes and environmental factors is a key driver of research questions spanning multiple areas of psychology. A nascent area of research focuses on the utility of epigenetic marks in capturing this intersection of genes and environment, as epigenetic mechanisms are both tightly linked to the genome and environmentally responsive. Advances over the past 10 years have allowed large-scale assessment of one epigenetic mark in particular, DNA methylation, in human populations, and the examination of DNA methylation is becoming increasingly common in psychological studies. In this review, we briefly outline some principles of epigenetics, focusing on highlighting important considerations unique to DNA methylation studies to guide psychologists in incorporating DNA methylation into a project. We discuss study design and biological and analytical considerations and conclude by discussing interpretability of epigenetic findings and how these important factors are currently being applied across areas of psychology.



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Contents

THE EMERGING INTERSECTION OF EPIGENETICS AND PSYCHOLOGY	460
DNA METHYLATION: AN ACCESSIBLE EPIGENETIC MARK FOR HUMAN PSYCHOLOGICAL STUDIES	461
Biology of DNA Methylation	462
Locations of DNA Methylation	463
DNA Methylation Technologies	464
CONSIDERATIONS: STUDY DESIGN	465
Candidate Genes Versus Epigenome-Wide Studies	465
Effect Size	465
Central Versus Surrogate Tissues	466
Timing of Biological Sampling	467
CONSIDERATIONS: BIOLOGICAL	468
Sex	468
Genetic Variation and Ethnicity	468
Age	469
CONSIDERATIONS: ANALYSIS	469
Types of Genome-Wide DNA Methylation Analyses	469
DNA Methylation Data Processing	470
Cell Type Differences	471
Verification, Validation, and Replication	473
Data Access	474
IMPLICATIONS OF DNA METHYLATION FINDINGS	474
Relationship Between DNA Methylation and Gene Expression	475
Epigenetic Age	476
Epigenetic Inheritance Across Generations	476
APPLICATION TO CURRENT AREAS OF PSYCHOLOGY	476
Developmental Studies	477
Candidate Gene Studies	477
Psychological Disorders	478
CONCLUDING REMARKS	478

THE EMERGING INTERSECTION OF EPIGENETICS AND PSYCHOLOGY

At the crux of psychological research is the question of how genetic predisposition and salient experiences together mold human behavior and psychological development. Epigenetic marks, a set of modifications to DNA and its packaging that can influence gene expression but do not alter genomic sequence, are hypothesized to mediate this interplay of genetic variation and experience, offering a potential avenue for investigating this fundamental question of how we become who we are. Consequently, the scientific community and the public alike have become interested in the role of epigenetics in developmental and psychological processes.

In contrast to DNA sequence, which is set at conception and is, for the most part, static across the lifespan, epigenetic marks undergo dramatic changes during the natural course of development. Variations in the epigenome, defined as the combination of epigenetic marks across the

genome, begin with the fundamental role the epigenome plays in cellular differentiation during embryogenesis, leading to distinct epigenetic profiles in particular cells and tissues. Although some epigenetic marks, especially those involved in determining cell fate, are highly stable, many continue to change over the life course in response to external cues from the surrounding environment. Moreover, epigenetic marks also appear to be a mechanistic overlay to the genome, molding genetically guided developmental plasticity processes in response to key signals. Epigenetic marks may thus provide a molecular basis for (a) the enduring effects of early life exposures via biological embedding and (b) the convergence of genetic and environmental variation in the manifestation of phenotypes (Boyce & Kobor 2015, Hertzman 1999, Meaney & Ferguson-Smith 2010). Given this role of epigenetic marks in orchestrating a delicate balance between persistence and plasticity across development, the study of these marks has become a prominent theme in health, clinical, and developmental psychology and related fields like behavioral neuroscience.

The growing interest in epigenetic processes as compelling developmental mechanisms is amplified by the increasing affordability of epigenetic technology, particularly in terms of one specific epigenetic modification, DNA methylation (DNAm). The number of large-scale projects incorporating DNAm has grown rapidly, yielding unprecedented opportunities for interrogation of DNAm in human populations. Rather than covering the theoretical reasons to investigate epigenetics, the purpose of this article is to contextualize existing epigenetic research in psychology in terms of the current available technologies and methodological advances. The unique aspects of DNAm biology that make it so fascinating also translate to a complex set of methodological considerations. Thus, after presenting a brief overview of epigenetics in general and DNAm in particular, we dive deeply into the world of epigenetic methodology before commenting on where current psychology research stands and how it can improve.

DNA METHYLATION: AN ACCESSIBLE EPIGENETIC MARK FOR HUMAN PSYCHOLOGICAL STUDIES

Epigenetics refers to modifications of DNA and DNA packaging that alter the accessibility of DNA and potentially regulate gene expression without changing the sequence of DNA itself. DNAm is the most highly studied epigenetic mark in human population studies, but other epigenetic factors include noncoding RNAs, histone variants, and histone tail modifications (Henikoff & Greally 2016). Together, these modifications are coordinated to regulate access to DNA by a variety of factors that control gene expression and cellular phenotype. DNAm is the most commonly studied in human populations for two major reasons: It is easily quantifiable, and it is relatively stable and, thus, does not require complex processing of samples after collection. Other epigenetic marks are more difficult to study, as they require special handling of samples or large amounts of sample.

One of the main roles of DNAm is in cellular differentiation. As stem cells divide and gradually differentiate into specific terminal cell types, DNAm patterns become increasingly cell type specific. This pattern of specification, which explains how cells with the same genetic sequence, such as neurons and white blood cells, have very different functions, was originally hypothesized by Waddington (1959). Landscapes of DNAm are, thus, highly divergent between cell types, with cells from similar lineages showing more similar DNAm profiles (Christensen et al. 2009, Ziller et al. 2013). Thus, in contrast to genetic information, DNAm is highly tissue specific. This trajectory of differentiation is reflected in widespread changes in DNAm over human development and suggests the possibility of windows of opportunity during which DNAm is changeable and particularly sensitive to environmental insults (**Figure 1**).

Notably, like gene expression and other traits, DNAm is also heavily influenced by genetic variation (Gutierrez Arcelus et al. 2013). Thus, the genome and environment together can sculpt

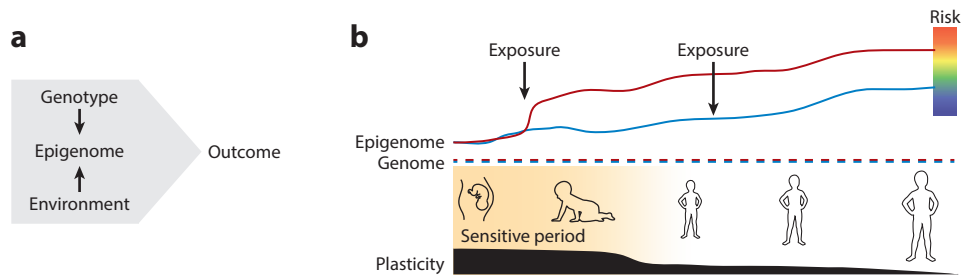


Figure 1

Conceptual outline of how DNA methylation can function as a mechanism of biological embedding. (a) The epigenome is sculpted by both the genome and the environment, and, together, these three factors can influence health and behavior outcomes over developmental time. (b) Example of how the epigenome could be altered during sensitive periods and result in risk. The blue individual and red individual have slightly different genomes (*dotted line*), and their epigenomes (*solid lines*) begin to diverge early in development due to genetic and environmental differences. During a sensitive period of heightened plasticity (*orange box*), the red individual is exposed to a particular environmental effect (*black arrows*), which alters their epigenome. Later in life, when the blue individual is exposed to the same environment, their epigenome is not altered, as they are out of the sensitive period.

DNAm across the lifespan, which is part of the reason why DNAm is such a compelling potential mechanism of biological embedding.

Biology of DNA Methylation

In humans and other vertebrates, DNA becomes methylated primarily on cytosine (C) nucleotides that are followed by guanine (G) nucleotides, which, in sequence, are referred to as CpGs. Methyl groups can also be found on cytosines in other contexts (non-CpG DNAm; see **Figure 2a**), although at much lower levels (Guo et al. 2013, Ziller et al. 2011). Critical enzymes called DNA methyltransferases (DNMTs) are responsible for the deposition of methyl groups at CpGs in DNA (Bestor 2000, Christensen et al. 2009). Because CpGs are palindromic in the double-stranded DNA, methyl groups are added symmetrically (**Figure 2a**). DNAm deposition can be *de novo*, when a completely unmethylated CpG becomes symmetrically methylated, but it is also required during cell division to ensure replication of DNAm patterns across generations of cells. When a cell divides, the newly replicated DNA strand contains only unmethylated CpGs, so DNMTs are required to recognize that a CpG is asymmetrically methylated and methylate the new daughter strand. Different DNMTs are responsible for each of these functions.

Together, these enzymes are responsible for DNAm being heritable from cell to cell during division. When cells divide, the action of DNMTs ensures that the epigenetic pattern from the mother cell is faithfully replicated in the daughter. Important aspects of epigenetic programming, such as cell lineage markers, are thus maintained across cell divisions. Interestingly, these mechanisms also allow for the cell-to-cell transmission of epigenetic patterns associated with the cell's past exposures—they create a form of cellular memory that can be passed along to daughter cells. It is these patterns that can be detected in studies examining associations between current DNAm and exposures or events in the past.

For many years, methylated cytosine was considered to be the most important modification to DNA involved in cellular memory, but recent research has shed light on other modifications, including 5-hydroxymethylation (hmC), which occurs in the same CpG context (Wu & Zhang 2014). The role of hmC is unclear, but it is present in the mammalian brain at levels 5–10 times

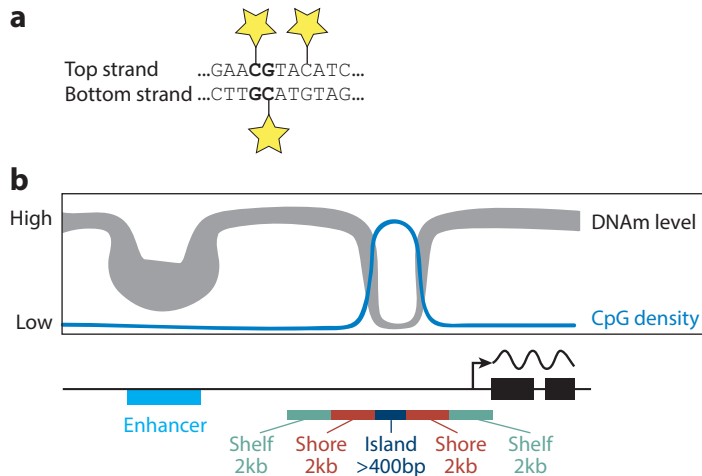


Figure 2

Locations of DNA methylation (DNAm) by genetic sequence and genomic region. (a) Methyl cytosine (indicated by a star) is present symmetrically on both DNAm strands at CpG dinucleotides (*left*) but on only a single strand if present at other cytosines (*right*). (b) Schematic of gene region with enhancer (*light blue*), transcription start site (*arrow*), and exons (*black rectangles*). The CpG island with shores and shelves is indicated below, with typical region sizes shown. Above is a schema of average CpG density (*blue line*) and typical DNAm level (*gray line*; thicker indicates more variability).

higher than in any other tissue (Ficz et al. 2011, Jin et al. 2011, Song et al. 2013, Wen et al. 2014). Importantly, many common methods of measuring DNAm do not differentiate methylcytosine from hydroxymethylcytosine, so it is critical to consider the presence of hydroxymethylcytosine when interpreting results from these types of assays (Stewart et al. 2015, Yu et al. 2012).

Locations of DNA Methylation

DNAm is not present uniformly across the genome, partly because of the relative scarcity of CpGs compared to other nucleotide combinations. Methylated cytosines, which are primarily observed in CpGs, are vulnerable to mutation into thymines, so it is hypothesized that they have been replaced by thymines over evolutionary time and thus are now found at lower than expected levels (Antequera 2003). Areas of comparatively high CpG content have been termed CpG islands; these islands tend to be unmethylated compared to nonisland CpGs and are found associated with approximately 70% of known gene promoters (Illingworth & Bird 2009, Weber et al. 2007). Flanking these regions of high CpG density and low DNAm are CpG island shores, which are usually defined as 2-kilobase regions on either side of the island (**Figure 2b**) (Irizarry et al. 2009). Shores tend to be more variable and more highly methylated than CpG islands and, thus, are more often of interest in population epigenetic studies. Beyond the shores are shelves, which cover an additional 2 kilobases flanking the shores. In total, there are approximately 28 million CpGs in the genome, but less than 10% are found in CpG islands. Because nonisland CpGs tend to be methylated, 60–80% of CpGs in the genome are methylated, and many are in repetitive sequences (Smith & Meissner 2013). The position of a CpG relative to a gene or other genomic feature is fundamental to its role in regulation of gene expression and will be discussed at length in the section titled Relationship Between DNA Methylation and Gene Expression.

DNA Methylation Technologies

DNAm can be measured in a number of different ways. In particular, the ability to make quantitative measurements, affordability, and scalability differ greatly between some of these methods and are important considerations for psychology studies (for reviews, see Bock 2012, Rivera & Ren 2013). There are two broad categories of methods to assess DNAm: pull-down based and bisulfite based. Pull-down-based methods rely on immobilized antibodies or proteins that recognize and bind to methylated cytosines, resulting in a pull down of methylated DNA, which is then sequenced to identify the regions where DNAm was found. Although these are less specific and less quantitative than the bisulfite methods discussed below, they are unbiased and can be powerful for exploratory purposes.

The other, more common, method of measuring DNAm is to use sodium bisulfite to convert DNAm information into differences in genetic sequence information, which can be easily quantified using well-established sequencing tools. Treatment with sodium bisulfite converts unmethylated cytosines into thymine (T), whereas methylated cytosines are protected and remain intact. Comparing the number of CpGs remaining to the number that converted to TpGs (thymine–guanine pairs) provides a quantitative measure of the proportion of DNA molecules methylated at that CpG position.

Within the four most common bisulfite sequencing methods, the main distinguishing feature is the number of CpGs that can be assessed. Small numbers of neighboring CpGs (tens to hundreds) can be quantified using technologies like pyrosequencing. Moderate numbers can be measured using targeted panels of up to a few thousand CpGs on a next-generation sequencer (Taylor et al. 2007). Both of these techniques are best used when the targeted genomic region(s) are known and limited, for example, when there is a small set of candidate genes. For a broader picture of DNAm in the cell and for discovery of novel genomic regions associated with a particular phenotype or condition, it is necessary to use a technique like large-scale sequencing or a microarray. Sequencing can be performed on the whole genome, but the relatively small number of CpGs, combined with the high cost of sequencing, means that this method is costly and bioinformatically challenging unless sequencing is targeted to CpG-containing regions using a technique like reduced representation bisulfite sequencing (RRBS) or CaptureSeq (Gu et al. 2011; Libertini et al. 2016a,b; Ziller et al. 2013, 2016).

The most common method for measuring DNAm is the commercially available microarray. These arrays hybridize bisulfite-treated DNA to immobilized probes that recognize specific regions of the genome and stain them with fluorescent labels to quantify methylated versus unmethylated DNA. Because microarrays are limited in the number of sites they can assess, they lack the true genome-wide measurements of whole-genome bisulfite sequencing, but their relatively low cost, their high reproducibility and reliability, and the plethora of existing data make them extremely attractive.

The primary source of DNAm microarrays is Illumina (based in San Diego, California); it began its production with the GoldenGate array in 2007, which measured approximately 1,500 CpGs (Bibikova et al. 2006). Since then, Illumina has released three new arrays, each larger than the previous one. The 27k, with approximately 27,000 CpGs, was released in 2009; the 450k, with approximately 480,000 CpGs, was released in 2011; and the newest model, the EPIC array, with approximately 860,000 CpGs, was released in 2016 (Bibikova et al. 2009, 2011; Moran et al. 2016). It should be noted that even the EPIC array is not truly epigenome wide, as it still covers less than 5% of CpGs present across the genome. Between 2007 and the present, methods and strategies for analyzing the data being generated by these arrays have been published, tested, and refined, as is discussed in detail in the following sections.

CONSIDERATIONS: STUDY DESIGN

The approach to incorporating DNAm should be informed by both the unique characteristics of DNAm data and the specific research question. In this section, we discuss a few key areas of study design specific to DNAm that are relevant to considerations of sample size, planned analyses, and inferences that can be made from DNAm findings.

Candidate Genes Versus Epigenome-Wide Studies

Early DNAm studies, particularly those conducted before the advent of genome-wide DNAm techniques, examined specific candidate genes hypothesized to be associated with the variable of interest. These methods have waned in recent years in favor of more global approaches, particularly microarrays, due to the increased affordability and information content of these approaches. Candidate and epigenome-wide approaches, however, are not mutually exclusive.

For researchers making use of epigenome-wide strategies but interested in particular candidate genes, DNAm for an *a priori* set of candidates may be selected from the array data for hypothesis-driven analyses. Data for these candidate genes would be isolated from and assessed separately from the remainder of the DNAm array data; whole epigenome discovery can then proceed after candidate analysis has been completed.

Analyzing candidates separately from the remainder of the whole genome data has the advantage of lower penalties for multiple testing. To take advantage of this multiple test correction benefit without adversely biasing or skewing the analysis, it is essential that the list of candidate genes be determined in advance. Previous studies on a variable of interest that examined DNAm, gene expression, or even genotyping are an excellent start, but it is also worthwhile to hypothesize and investigate potential molecular mechanisms implicated by basic research. The number of candidate genes identified can be flexible, but multiple test penalties should be considered, as should the total number and position of CpGs assigned to the gene of interest available on the array. For example, if gene *A*, which may be involved in the variable of interest, has 2,000 CpGs present on the array, then this will significantly degrade the benefits of reducing the number of tests. In these cases, and where sample sizes limit power, it may be beneficial to select a few CpGs from important regulatory regions, such as transcription factor binding sites or enhancers, rather than testing all gene-associated CpGs.

It is also important to note that there are substantial advantages to epigenome-wide DNAm analysis aside from the opportunities for discovery of novel associations. With array DNAm data, it is possible to identify other important biological variables that may not have been measured. These include (a) ethnic group, using methylated sites that are highly predictive of ancestry (Rahmani et al. 2016); (b) relative proportion of cell types in each sample (if quantified in a heterogeneous tissue), based on methylation of a few hundred reference CpGs (Esposito et al. 2016, Guintivano et al. 2013, Houseman et al. 2012); and (c) genetic relatedness of samples from individuals, using the single nucleotide polymorphisms (SNPs) present on the DNAm arrays. As discussed in the section titled Considerations: Biological, these variables are important for properly controlling DNAm analyses, and, thus, the ability to extract them from array data is a significant bonus.

Effect Size

An important consideration when embarking on a study involving DNAm is that the effect sizes will likely be quite small. Effect sizes in DNAm studies are partly constrained by how and where DNAm is measured. For example, in a single cell, a particular CpG is present only twice (once on each member of a chromosome pair), so the DNA can be 0%, 50%, or 100% methylated. When

assessing a participant's DNA sample, however, hundreds or millions of cells are measured at the same time. Thus, a change in DNAm at a small subset of cells will, by necessity, be reflected as a small but dimensional change in overall DNAm of the whole biological sample. Small effects can also be attributed to the nature of methylation at the regions in the methylome that are commonly targeted. CpG islands and promoters typically have very low DNAm levels; thus, they have a low dynamic range over which they could exhibit DNAm changes.

The typical effect sizes, including those of DNAm findings that have been extensively validated, generally do not exceed 10% in terms of the mean difference in proportion of methylated DNA strands between groups of individuals (Breton et al. 2017). The mean difference is often referred to as a delta beta, typically calculated as the mean difference between groups or the range in a certain interval for continuous variables. For instance, replicated associations between smoking exposure and DNAm both in adults and in infants exposed in utero typically range in effect size from 1% to 10% (Gao et al. 2015, Joubert et al. 2016). DNAm differences for the broader, noisier, or less objective exposures (e.g., socioeconomic status) common in psychology are expected to be comparable or even smaller. Researchers must therefore carefully consider the variability in a targeted region and assume small effect sizes to design studies with adequate power (see the section titled Verification, Validation, and Replication).

In addition, combining the small effect sizes with possible technological or biological variation means that it can be difficult to distinguish true signals from noise. One often-recommended method that decreases the likelihood of false positives is establishing an a priori threshold to consider a finding biologically significant. An arbitrary delta beta of 5% (meaning that 5% of DNA tested gained or lost DNAm at that locus) is common. However, many findings in exploratory studies linking DNAm to exposures in psychology do not meet this threshold, and smaller effect sizes (approximately 2%) have been extensively validated in large cohorts and shown to have downstream effects on gene expression (Breton et al. 2017).

Central Versus Surrogate Tissues

In the context of human studies, the tissue specificity of DNAm creates a significant challenge for the use of surrogate tissues in cases where the primary tissue of choice is not available. This is particularly relevant to psychological studies, where, in many cases, DNAm within the brain might be of ultimate interest but samples of the brain can only be collected postmortem. Postmortem tissues have been useful in many studies, but for studies of living participants, accessible peripheral tissues, such as saliva, buccal epithelial cells collected from a cheek swab, or blood collected by venipuncture or a finger prick, are the only viable options (Davies 2009, Lowe et al. 2013).

The challenge for studies that use peripheral tissues as surrogates is determining whether DNAm differences associated with phenotypes in the surrogate tissue are reflecting parallel changes in the tissue of interest. For many studies focused on behavior, it is thus imperative to rigorously ascertain the extent to which peripheral epigenetic patterns reflect those in the brain. Recent studies on the concordance between central and surrogate tissues have shown mixed patterns, in which some sites are highly concordant across tissues but others are discordant (Davies et al. 2012, Farré et al. 2015, Hannon et al. 2015). Some reports have suggested that the most concordant sites between tissues are more likely to be genetically regulated (Edgar et al. 2017a, Hannon et al. 2015). A few of these studies have created resources that allow patterns of DNAm at specific CpGs of interest to be compared between blood and brain, including BeCON (<https://redgar598.shinyapps.io/BECon/>), a recently published resource from our group (Edgar et al. 2017a, Hannon et al. 2015). These are excellent resources for determining whether findings in blood can be expected to be similar in the brain. Parallel efforts for other tissues such as saliva and cheek swab will be key to future research.

In other cases, accessible biological samples such as blood may, in fact, be extremely informative, with DNAm in these tissues more directly reflecting the exposure of interest. In particular, for studies assessing the stress response and its effects on inflammation, blood is, in fact, the correct primary tissue (Kim et al. 2016, Ligthart et al. 2016). In these situations, identifying functional associations between exposures and DNAm is more likely because the effect is being observed in the tissue of interest.

Timing of Biological Sampling

The inferences that can be made from a DNAm finding depend on the time point at which measurement of DNAm occurred in the course of a study design. For this discussion, it is helpful to distinguish between studies targeting DNAm as an outcome of an exposure, as a predictor of a psychological condition, or as a mediator (i.e., a potential mechanism bridging an earlier exposure to a behavioral outcome).

In developmental research, DNAm is commonly assessed as an outcome of an earlier environmental exposure. Despite the apparent temporal precedence of exposures before DNAm measurement, without longitudinal repeated measures, the inferences drawn in this case must be primarily correlational. DNAm patterns at one time point, accompanied by either recollected measurements of an early exposure or an exposure that was measured in a sample followed prospectively, will not necessarily reflect a pattern attributable to the exposure of interest because environments are often highly confounded. To infer causality, DNAm should be measured a minimum of once before the exposure and once after (if the exposure is postnatal).

In the case of studies with a focus on DNAm as a predictor of future psychological conditions, assessing DNAm at multiple time points may not be as critical, depending on the hypothesis being tested. If DNAm patterns are hypothesized to be a biomarker of risk for developing a disorder at a later time point, it is adequate to obtain one snapshot of DNAm measured at a time point that precedes the typical onset of the disorder. Obtaining two time points of DNAm still offers an advantage, however, if there is interest in biological changes that may accompany the transition to a psychological disorder. Comparing DNAm pre- and post-disease onset with two time points could identify such a pattern (although whether DNAm plays a causal role would still be unclear).

In psychological science, assessment of statistical mediators is commonplace. Many studies conceptualize DNAm as a biological mechanism that links environmental exposures to subsequent outcomes. It is important to note that time ordering is necessary for statistical mediation analysis to distinguish a mediator from a merely confounding influence and is still not sufficient for causal inference (MacKinnon et al. 2000). As mentioned above, DNAm may reflect stable a priori differences and may be attributable to inherent differences between an exposed and unexposed group. Thus, the preferable design to support a mediation model would be assessment of DNAm at both a pre-exposure time point and a time point between exposure and the outcome. Moreover, random assignment of an exposure obtained, for example, through a randomized controlled trial in intervention research is the only means of estimating a true causal effect of an exposure on DNAm, and this randomization must be done in the context of an adequately powered study. Unless a mediator, in this case DNAm, is also randomly assigned, estimating its causal effect on a psychological disorder is not possible.

Finally, although it is ideal to collect more than one time point of DNAm data, it is clearly a costly endeavor. Researchers have to weigh the trade-off between a larger sample size and a larger number of time points of DNAm; their decision will depend on the larger research question and strategy. Regardless of the design selected, it is important for researchers to acknowledge the limitations of their study and to approach mechanistic interpretations cautiously.

CONSIDERATIONS: BIOLOGICAL

Once study-specific decisions such as sample size, study type, and tissue have been made, it is important to consider some unique biological properties of DNAm data and how they will influence data collection and analysis. Because DNAm plays an important role in development and differentiation and has a close relationship with genetic variation, these factors can affect DNAm study results adversely if they are not incorporated into study design and analysis.

Sex

Psychological conditions or behaviors can often be sex specific, resulting in substantial interest on the part of researchers in identifying sex-specific DNAm patterns. Sex chromosome composition is a major driver of genome-wide DNAm patterns, rendering these analyses highly interesting. DNAm is involved in the inactivation of one of the two X chromosomes in females. The silent X chromosome is tightly packaged and heavily methylated, resulting in a highly differential pattern of X chromosome methylation between males and females. Because of this important difference, the X and Y chromosomes should be analyzed separately from the autosomes, as they require different normalization and analysis strategies (Cotton et al. 2015).

Beyond the X chromosome, sex in general is also a determinant of DNAm pattern on the autosomes. The mechanism by which these sex-specific differences arise is unknown but likely reflects the many physiological and developmental differences between males and females. This sex-specific variation, then, is of particular interest to researchers looking for DNAm patterns associated with sex-specific psychological conditions or phenotypes. Incorporating sex into analyses by, first, attempting to balance sex between groups; second, including it as a covariate; and, possibly, third, including it as a moderator in downstream analyses, is critical for DNAm studies.

Genetic Variation and Ethnicity

DNAm is associated with ethnic backgrounds due to shared genetic ancestry as well as cultural and environmental commonalities. The relationship between DNAm and genetic variation is complex, but there are specific sites of DNAm that are highly associated with nearby genetic variants such as SNPs, referred to as methylation quantitative trait loci (mQTLs) (Banovich et al. 2014, Fraser et al. 2012, Gutierrez Arcelus et al. 2013, Hannon et al. 2016, van Dongen et al. 2016). mQTLs may be tissue and developmental stage specific and may vary widely across a population, even within an ethnic group (Teh et al. 2014). Genetic variation is only one of several means by which ethnicity is related to DNAm. Cell type differences, diet, lifestyle, or habitat, as demonstrated by the examples below, also contribute to ethnicity-driven patterns in DNAm. Researchers can attempt to account for these factors by controlling for ethnicity in DNAm studies.

Recent work has isolated genetic effects from environmental and cultural influences on DNAm. One study assessed DNA sequence and epigenomic variation in two African populations with different current habitats, as well as historically different lifestyles (hunter-gatherers versus farmers) (Fagny et al. 2015). Both current habitat and historical circumstances had impacts on variation in DNAm: Variation was accounted for by historical population differences corresponding with nearby genetic variation. The functions of genomic regions implicated in historical ancestry related to developmental processes, whereas current habitats implicated genomic areas with cellular and immune functions. Similarly, in a study of individuals from diverse Hispanic backgrounds, self-identified ethnicity and genetically determined ancestry each accounted for common as well as distinct variation in the methylome. Self-identified ethnicity but not ancestry overlapped with prenatal exposure to smoking, suggesting that culturally identified ethnic groups reflect exposures that may not be accounted for if only genetic ancestry is considered (Galanter et al. 2017).

Age

DNAm is not stable over the lifetime; rather, it has been shown to change with age, in some cases becoming more variable over time and in others demonstrating tight associations with chronological age. This was first observed in identical twins, where young twins were epigenetically highly similar, but older twins became more and more different (Fraga et al. 2005). This observation prompted the epigenetic drift hypothesis, in which environmental and stochastic changes over the lifespan are embedded into the genome and contribute to increasing diversity over time (Teschendorff et al. 2013b).

In addition to the interindividual divergence in DNAm patterns, there are common age-related patterns in DNAm changes that occur across individuals. All tissues examined to date have shown an overall decrease in DNAm with age, although some sites gain DNAm (Bell et al. 2012, Bjornsson et al. 2004, Boks et al. 2009, Florath et al. 2014, Hannum et al. 2013, Horvath et al. 2012, Johansson et al. 2013, Lister et al. 2013, Weidner et al. 2014). Changes in DNAm with age have been extensively reviewed (for further information, see Issa 2014, Teschendorff et al. 2013b, Zampieri et al. 2015).

The associations between DNAm and age are important to consider when beginning a study that will measure DNAm across participants. If the study sample includes a wide range of ages, it is important to attempt to balance ages across groups and to control for chronological age in analyses. Another age-related epigenetic phenomenon, the epigenetic clock, is discussed in the section titled Epigenetic Age.

CONSIDERATIONS: ANALYSIS

The biological and study design considerations described above can result in challenges for DNAm analysis. The effect sizes of some of these variables, particularly ethnicity, age, and sex, on DNAm can be larger than the effect sizes observed for an exposure or condition of interest in psychological studies. Thus, it is particularly important to check for and statistically correct confounders, as they can easily overwhelm true signal and inflate false positives. Conversely, the statistical noise associated with these variables can mask subtle associations with variables of interest even when they are not confounded with it, resulting in an inflation of false negatives. It is recommended that, in any study where age, sex, and ethnicity are not either uniform or balanced across groups, these variables be, at minimum, included as covariates in analyses.

In addition to the need to control confounding variables, DNAm data has unique characteristics that influence its analysis. DNAm data is often presented as a beta value approximating percent methylation. Because most assays measure DNAm across thousands or millions of cells, the beta value represents the average level of DNAm across all of these cells. Beta values are biologically meaningful but can be statistically problematic, as they have low variances at methylation values near 0 or 1. For that reason, statistical analysis is often performed on M values—log transformed methylation values—but results are typically reported as the more biologically relevant beta values (Du et al. 2010).

Types of Genome-Wide DNA Methylation Analyses

As DNAm studies have become more frequent, analysis methods have been designed to take advantage of the opportunities of this particular data type. DNAm data analysis has borrowed methods from both gene expression research and genome-wide association studies, and early studies primarily used epigenome-wide association studies along with assessments of global DNAm

that measured mean DNAm or DNAm at repetitive elements in the genome (Baccarelli et al. 2010, Bollati et al. 2009, Rakyan et al. 2011). These analyses are still popular but are now complemented by more complex methods.

Analyses often include an association study identifying specific CpGs or groups of CpGs that are associated with a specific variable of interest. Methods used in this type of analysis include multiple linear regression or correlation analyses. Recent advanced methods have assessed more complex groupings of CpGs; these methods fall into two main categories based on whether they group CpGs in physical neighborhoods or by similarities in DNAm pattern. The former includes methods like DMRcate and Bumphunter, which incorporate the genomic location information for CpGs to identify regions of differential DNAm (Jaffe et al. 2012, Peters et al. 2015). These region-based methods are powerful but require caution for two reasons: First, in cases where variables have small effect sizes, these methods can show bias toward less variable, more CpG-dense regions. Second, many of these methods do not take into account the possibility that multiple functional elements may underlie a cluster of CpGs, which may result in erroneously grouping CpGs that are not functionally related to one another. The other category discovers CpGs with similar DNAm patterns regardless of genomic location, as methylation of CpGs at disparate locations may be functionally related due to the folding and packaging of chromatin. These methods include weighted gene correlated network analysis and functional epigenomic modules (Jiao et al. 2014, Langfelder & Horvath 2008).

In addition to associations, recent work has focused on DNAm variability as a potential marker. These assays determine whether interindividual variability, either at specific probes or across the epigenome, is different between groups. Higher variability has been associated with depression in discordant twins, as well as with type 1 diabetes (Dempster et al. 2014, Paul et al. 2016); however the consequences of altered variability in DNAm are still poorly understood.

DNA Methylation Data Processing

Raw DNAm data must be extensively processed prior to analysis. To monitor the effectiveness of these steps, DNAm data is often assessed using principal components analysis (PCA), a strategy to reduce many variables to a set of independent components that account for maximal variability in the data. By condensing the data from thousands of probes to a manageable number of principal components, statistical associations between these components and technical and biological variables can be observed to confirm that data processing steps are performed correctly (**Figure 3**). In **Figure 3**, which uses cheek swab samples, PCA indicates the relative strengths of associations with technical (batch) and biological (sex, ethnicity, age, and cell type) variables. This and the following sections will outline the steps involved in reducing these confounders. Below, we discuss each stage of data processing and refer back to **Figure 3** to highlight how successful data correction is confirmed.

The initial preprocessing steps deal with technical variables inherent in the DNAm microarray, outlined in **Table 1** (Bibikova et al. 2011, Fortin et al. 2016). First, detailed examinations of the genomic regions assessed on the DNAm arrays have shown that a subset of probes on the array are technically unreliable, and lists of such probes that should be excluded from analysis have been published (Y.-A. Chen et al. 2014, Pidsley et al. 2016, Price et al. 2013). Second, many CpGs present on the array are invariable across samples, particularly within a tissue. An empirically determined set of these invariant CpGs has been identified and can be removed from analysis to minimize multiple hypothesis testing (Edgar et al. 2017b). Third, DNAm data can show batch effects, an issue common to microarrays and sequencing platforms. Batch effects can be detected and corrected, if found, using methods such as ComBat or sva (Leek et al. 2012, Teschendorff

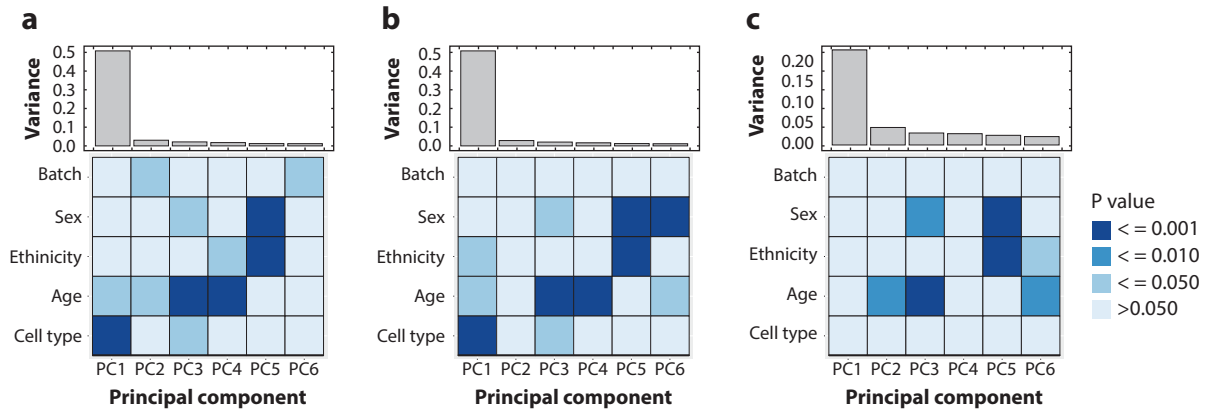


Figure 3

Principal components analysis of DNA methylation (DNAm) data indicating association of technical and biological variables with principal components. The DNAm data were derived from cheek swabs, which can include both buccal epithelia and white blood cells, so the relative proportions of buccal epithelia were calculated using the reference-based methods described in the section titled Cell Type Differences. In each case, the top bar plot indicates the proportion of variance explained by each of the top six principal components, and the bottom heat map indicates the p-value of analysis of variance (ANOVA) (for batch, sex, ethnicity, and age) or Spearman correlation (for cell type). (a) Raw data shows strong associations with each variable, with cell type responsible for approximately 50% of the variance. (b) After correction for batch, no principal components are still associated with technical batch, but 50% of variance is now associated with cell type. (c) After correction for cell type, the top principal component has less variance (approximately 20%) and is no longer associated with cell type. Associations with age, sex, and ethnicity remain strong and should be corrected for either prior to or during analysis.

et al. 2011). In **Figure 3b**, we demonstrate the use of PCA to assess the success of batch correction, showing that variability due to the technical batch effect has been removed.

Cell Type Differences

As described above, DNAm plays an important role in the development and differentiation of specific cell types. Stem cells exhibit specific DNAm and gene expression patterns, which are further resolved as the cells differentiate (Álvarez-Errico et al. 2014, L. Chen et al. 2014). This results in terminally differentiated cell types with highly cell type-specific DNAm patterns (Guintivano et al. 2013, Reinius et al. 2012). In fact, cell type within a tissue is the second-biggest contributor to DNAm variation, after tissue type (Farré et al. 2015). The example in **Figure 3** shows the extent to which cell type proportions are associated with DNAm profiles, as more than 50% of the variance in the data is due to interindividual differences in buccal cell proportions (shown in **Figure 3a,b** but corrected in **Figure 3c**). If this data had been analyzed without correcting for cell types, it would be unclear whether any significant findings were due to true DNAm differences between groups or simply to differences in cell composition.

In the brain, for example, neurons and glia show very different DNAm profiles (Guintivano et al. 2013, Lister et al. 2013). This means that, if two samples with different proportions of neurons are compared, then the differences between the samples will primarily reflect those differences in cell type, rather than any underlying condition or phenotype. It is thus important to consider tissue sampling across participants to minimize interindividual differences in cell counts. Cell counts are often not collected when tissues are stored due to labor intensiveness and cost, but, fortunately, it is possible to predict underlying cell type differences for brain tissue, as well as for some of the

Table 1 Steps in data cleaning and processing for DNA methylation data from Illumina microarray platforms

Step	Description	Common tools
1. Raw data	Data is generated from the scanner and extracted as IDAT files	Illumina scanner software
2. Background subtraction, color correction	Basic normalization removes background signal and equalizes the intensities of the two colors used on the arrays	Illumina Genome Studio or Minfi R package (Aryee et al. 2014)
3. Probe filtering	Probes that are unreliable or unnecessary are removed. These can include: <ul style="list-style-type: none"> ■ Probes with high within-sample variability or insufficient signal ■ Probes that are known to hybridize to multiple sites in the genome ■ Probes found in repetitive regions ■ Probes on the X and Y chromosomes (see the section titled Sex) ■ Probes with little or no interindividual variability (optional) 	Additional annotations (Y.-A. Chen et al. 2014, Edgar et al. 2017b, Price et al. 2013)
4. Normalization	Normalization equalizes the ranges of the two probe types on the array	BMIQ, wateRmelon R package, Minfi R package (Aryee et al. 2014, Pidsley et al. 2013, Teschendorff et al. 2013a)
5. Batch correction	Batch correction removes any effects of the batch, including chip, plate, or row	ComBat function in sva R package (Teschendorff et al. 2011)
6. Final data	At this point, data can be analyzed or corrected for known confounders (e.g., cell type, sex, ethnicity)	See Jones et al. (2015b)

more common surrogate tissues: saliva, blood, and cheek swab (Guintivano et al. 2013, Houseman et al. 2012, Smith et al. 2015).

These methods are referred to as reference-based methods because they rely on reference profiles created from isolated cell types to infer the likely underlying cell composition (Houseman et al. 2012). Reference-based methods are limited, however, by the requirement for reference profiles for each component cell type, which means that they often cannot predict proportions for very infrequently occurring cell types or for tissues in which it is difficult to isolate cell types. The references may also be developmental stage specific, which could influence the accuracy of the predictions at different ages. To circumvent some of these limitations, reference-free methods correct for the effects of cell composition without actually predicting the cell counts (Houseman et al. 2012, 2014; Lam et al. 2012; Zou et al. 2014). However, it is much more difficult to assess the performance of these measures in reducing interindividual variability. The use of reference-based methods that predict cell types versus methods that correct for interindividual variability without using cell type references is still highly debated (Hattab et al. 2017; McGregor et al. 2016, 2017). Because excellent and well-characterized reference profiles for adult blood, brain, and saliva exist, these profiles are reliable and recommended for use in DNAm studies (Guintivano et al. 2013, Houseman et al. 2012, Smith et al. 2015). However, it has been shown that adult blood references do not work well on cord blood, for example, so for other tissues or developmental stages, it is important to consider both reference-based and reference-free methods (Yousefi et al. 2015).

In both cases, the method incorporates information on interindividual variability due to cell type, either directly, as in reference-based methods, or indirectly, as in non-reference-based methods, with the goal of controlling for this important type of variation (Jones et al. 2015b).

Adding further complications, the differences in cell type can themselves be related to the phenotype, as has occurred in a number of studies (Esposito et al. 2016, Jones et al. 2013, Liu et al. 2013). In those cases, the cell type differences are an important and interesting finding to be discussed, but they must be carefully controlled for if the goal is to find DNAm changes that are independent of cell type (Paul et al. 2016).

Verification, Validation, and Replication

Another consideration for DNAm studies is the sheer magnitude of measurements—bisulfite sequencing may record millions of CpGs, the 450k array measures over 480,000 sites, and the Illumina EPIC array measures over 850,000 sites. With this number of tests, it is essential to correct for multiple comparisons to avoid false positives, but typical methods can be overly conservative. Stringent multiple test correction, like Bonferroni $p < 0.05$, would require a nominal p-value less than 5×10^{-8} , so more often, a false discovery rate (FDR)-based method like Benjamini-Hochberg correction is used (Benjamini & Hochberg 1995). This method also has limitations, particularly the fact that the corrected p-value is based on the underlying distribution of nominal p-values, which can be complex to resolve across experiments if the underlying distributions are different. Partly for these reasons, some researchers are now using a very low nominal p-value (approximately 1×10^{-6}) or a higher FDR (approximately 0.1) and adding a second measure of biological feasibility, such as variability or range, to reduce the number of hits (Esposito et al. 2016, Ladd-Acosta et al. 2014, Lam et al. 2012). However, this loosening of statistical stringency makes verification, validation, and, especially, replication of DNAm findings particularly important.

In this context, verification refers to measuring DNAm through another method—often pyrosequencing of hits from array technology. This method occasionally has the added benefit of measuring new CpGs in the genome that are not on the array, adding new associations with the variable of interest. Validation refers to measuring the same relationship between the variable of interest and the hit in samples reserved from the initial study. Replication entails repeating the same analysis on completely independent samples (Michels et al. 2013).

Thus, when designing studies, it is important to budget and reserve samples to verify findings by another method, to either reserve a subset of samples for validation or recruit additional participants for that purpose, and to examine the literature closely for appropriate cohorts for replication. One important consideration, especially for replication in an independent data set, is the idea that methylation in the replication should be assessed in the same tissue and age range as in the original study, and the variables of interest should be assessed by common measurement. It might be challenging to replicate a finding discovered in blood samples in a cohort with cheek swabs, for example. Differences in tissue type or variable measurement can reduce the likelihood of replication.

Within the psychology landscape, reproducibility of results has become an issue at the forefront of discussion (see Open Sci. Collab. 2015). The risk for false positives is substantial for expensive methylation studies due to typically limited sample sizes, small effects, and, in the case of array-based data, the measurement of hundreds of thousands of data points. Collaborations and the development of research consortia can build the funding and resources required for larger sample sizes and concomitant replications.

As more DNAm data sets are generated, there are sufficient numbers of published findings to allow the application of meta-analysis. A number of consortia have been organized to combine cohort data with the goal of creating larger studies in which to validate and extend prior findings. Recently, a large meta-analysis validated previous findings, outlined above, on the connection between DNAm at genes in the aryl hydrocarbon receptor repressor (AHRR) pathway and prenatal exposure to cigarette smoke, and also discovered previously unknown associations

(Joubert et al. 2016). In the near future, meta-analyses will undoubtedly become more prevalent, which will help to confirm reliable results.

Data Access

Microarray, sequencing, and other types of data are frequently deposited to online repositories, and, indeed, upon acceptance of a manuscript, many biomedical journals require that such data be deposited so that it can be publicly accessed. It is in the best interests of researchers and funders alike that this deidentified data be shared widely, as it allows for effective replication of studies. Making use of this data for replication comes with its own challenges but is hugely advantageous to the field as a whole. Public access to data can be a sensitive issue, as participant data collected as part of a study can often be proprietary and personal, with associated ethical issues. It is thus important to verify that proper consent is in place when biological samples are collected to ensure that data can be anonymized and shared with the broader community. In cases where specific types of data cannot be shared, it is beneficial for researchers to be open to collaborations with others who may request specific analyses or reanalyses of data.

IMPLICATIONS OF DNA METHYLATION FINDINGS

Perhaps the most intriguing aspect of epigenetic studies in psychological science is the possibility of identifying biological mechanisms accounting for the effects of environmental exposures correlated with human behavior. However, it is important to approach epigenetic findings with caution and to resist the allure of jumping to mechanistic interpretations. At the present stage of human research, correlations between psychological conditions or environmental exposures are, indeed, correlational and do not necessarily imply a causal mechanism. Currently, associative epigenetic signatures are best understood as potential biomarkers until further investigation of molecular mechanisms discovers underlying functional effects.

The distinction between a DNAm finding being a biomarker versus a causal mechanism is one of the fundamental questions in epigenetics today and involves asking whether there is sufficient evidence to infer whether differences observed are cause or consequence (Ladd-Acosta 2015). It is important to note that determining actual causality in any association study is very challenging (see the section titled Timing of Biological Sampling). Mendelian randomization is one method that has been proposed to assess causality without the burden of longitudinal measurement. It uses genotype as a fixed variable from which to test causality but is possible only in cases where there is a known genetic variant that is uniquely associated with the variable of interest (Relton & Davey Smith 2012), an assumption that is rarely met with psychological phenotypes.

Without causal study designs or Mendelian randomization, it is still possible to make hypotheses as to whether an association between DNAm and a variable of interest might be functional or not and to target identified sites in downstream molecular experiments. The uncertainty of these interpretations should be presented, however, and mechanistic language should be limited in this presentation. Evidence that a psychological DNAm finding in a surrogate tissue such as blood or cheek swabs exhibits a similar relationship in brain tissue would be encouraging for future research. Also, prior evidence that DNAm at the region of interest is associated with gene expression changes or conformational changes to DNA would also increase confidence that further work might uncover a mechanism.

In cases where such evidence is not available, associations between DNAm and a variable of interest can still be relevant as biomarkers. These biomarkers can be used for identification purposes and can be particularly useful if they are marks of an exposure or event that can have

future health relevance. In that case, these marks could inform targeted interventions or lifestyle changes to alter future risk.

In both cases, signatures of DNAm that are associated with an environmental exposure, health, or behavior offer translational avenues. For associations for which evidence of mechanistic involvement is available, understanding the actual molecular mechanism is important for identifying novel therapeutics or treatments. Biomarkers can also be used to stratify populations for more targeted interventions or as early markers of intervention effectiveness (for an excellent review of the challenges of interpreting epigenetic findings, see Lappalainen & Grealley 2017).

Relationship Between DNA Methylation and Gene Expression

When analysis is completed and specific CpGs or groups of CpGs have been identified as being associated with a variable of interest, one potential next step is assessing whether the DNAm differences observed alter gene expression. This is not required, as DNAm associations can be valuable even if gene expression does not change, but is one way to assess possible outcomes. Although measuring RNA in addition to DNAm is ideal, RNA is often not collected, as it is more difficult to stabilize during biological sampling. It is possible to infer potential influences on gene expression from DNAm patterns, but this has two related complications. The first is determining how (or whether) to assign CpGs to a specific gene, and the second is determining what possible effects on gene expression the DNAm change might have; these effects depend on both the direction of the DNAm change and the specific location and function of the CpG.

Mapping CpGs to a specific gene is more difficult than it might appear, as CpGs might be found within or near a single gene or near multiple genes. Many systems have been created to annotate CpGs, particularly those on the 450k array, to specific genes, and these can vary widely (Y.-A. Chen et al. 2014; Edgar et al. 2017a; Farré et al. 2015; Price et al. 2013, 2016). It is also important to note that, in some cases, CpGs may not be annotated to a gene at all. This does not mean that the differential DNAm of that CpG has no outcome; instead, it is possible that it is involved in a relationship that has not yet been mapped.

Once the CpGs of interest have been mapped to genes, it is possible to hypothesize how the observed change might influence expression of those genes based on the type of genomic feature in which the change is observed. In general, genomic features like DNase hypersensitive sites, enhancers, and transcription factor binding sites are indicators that there might be an association with gene expression. A University of California, Santa Cruz website (<http://genome.ucsc.edu/cgi-bin/hgGateway>) is an excellent resource, as it has a useful visual interface; includes data from large-scale mapping projects like ENCODE, Roadmap Epigenome, and the 1,000 Genomes Project; and can be customized with user-generated data (Bernstein et al. 2010, Diehl & Boyle 2016, Meyer et al. 2013, Sloan et al. 2016). Generally, promoter CpG island-associated DNAm is weakly but negatively associated with gene expression, whereas gene body DNAm is weakly but positively associated (Gutierrez Arcelus et al. 2013, Schübeler 2015, Wagner et al. 2014). Other genomic features, like enhancers and insulators, have less clear trends (Kozlenkov et al. 2014, Ziller et al. 2013). Hypotheses regarding gene expression changes should be tempered, however, as there are many exceptions to these rules, and other underlying genomic features might influence expression in a gene-specific manner (Gutierrez Arcelus et al. 2013).

Finally, it is essential to examine the tissue-specific expression of any associated genes to determine whether and when genes are expressed. In some cases, DNAm differences may be found at genes that are not expressed in a particular tissue or are only expressed under specific conditions. DNAm patterns have been shown to prime cells for future responses and, in some cases, predict condition-specific gene expression, so it is important to investigate these issues (Lam et al. 2012).

Epigenetic Age

In addition to the above-described assessment of specific CpGs or groups of CpGs to a variable of interest, a recently developed tool provides an alternative use of DNAm data. Researchers had previously observed that DNAm levels were associated with chronological age, but in recent years, specific epigenetic clocks—patterns of DNAm that accurately predict chronological age across a population—have been published (Florath et al. 2014, Hannum et al. 2013, Horvath 2013, Weidner et al. 2014; for a review, see Jones et al. 2015a). Thus, in contrast to the epigenome-wide and candidate gene studies described above, an analysis of epigenetic age is calculated based on a specific set of CpGs, which are highly informative of age.

It has been hypothesized that these clocks represent a measurement of biological age, and, indeed, studies have shown that deviations of epigenetic age—that is, higher epigenetic age compared to chronological age—is associated with a variety of health conditions (Horvath & Levine 2015; Horvath et al. 2015, 2016; Levine et al. 2015; Marioni et al. 2015). Given the general interest in psychology in the lasting impacts of stress on the aging process, the use of epigenetic clocks for determining age acceleration has become particularly useful for testing hypotheses on the mechanistic links between psychological stressors and age-related disease. For instance, age acceleration has been predicted by cumulative lifetime stress, trauma, and harsh parenting (for a review, see Zannas et al. 2015), and these effects can be counteracted by supportive environments (Brody et al. 2016a,b; Miller & Sadeh 2014).

Epigenetic Inheritance Across Generations

A particularly intriguing feature of epigenetics in the developmental origins of health is the idea of epigenetic inheritance across generations. As mentioned above, DNAm is heritable from cell to cell (i.e., within one organism's lifespan), creating a memory of DNAm that reflects past exposures. The idea that this could be extended to human generations, i.e., that epigenetic changes can be inherited from parent to child, is very compelling and, simultaneously, complex.

The idea of inheritance across generations has been divided into two categories: intra- and inter- or transgenerational epigenetic inheritance (Miska & Ferguson-Smith 2016). The theory of intragenerational inheritance involves the idea that, during pregnancy, child DNAm patterns are altered by exposure to the maternal in utero environment, which is a product of the mother's lifetime exposures. These effects may plausibly be observed up to two generations after the exposure, as the germ cells of the exposed child, which will go on to make the next generation, are also exposed. True transgenerational inheritance requires transmission of epigenetic marks themselves to the next generation in the absence of the exposure. Transgenerational inheritance thus requires evidence of three-generation transmission through the female line, as the offspring of the third generation is the first to not directly encounter the in utero exposure. These distinctions have been covered in detail in an excellent review (van Otterdijk & Michels 2016). Correct usage of these definitions is essential to accurately describe how environmental influences propagate across generations. To date, there is evidence for intragenerational transmission of many environmental exposures but no true evidence of transgenerational inheritance in humans (Heard & Martienssen 2014, Joubert et al. 2016, Radtke et al. 2011).

APPLICATION TO CURRENT AREAS OF PSYCHOLOGY

Now that we have outlined the methodological and conceptual considerations in DNAm studies, we provide an overview of some existing issues in the psychology literature, in which epigenetic

analysis has become popular. We outline a few examples, reflecting on how the issues raised above have been addressed (or unaddressed) to date. We note that the intention in highlighting some examples is not to single out particular studies but merely to elaborate on methodological advancements, as the understanding of the issues discussed above has grown rapidly in the past few years.

Developmental Studies

The designs and goals of developmental studies incorporating DNAm are wide ranging, but tissue type and time ordering of sample collection are particularly relevant issues. Out of necessity, many studies assess DNAm in a common surrogate tissue, blood, years after an early environmental exposure. Far from being a limitation, in studies focused on the biological embedding of stress, blood is actually the tissue of interest. Specifically, psychological exposures are theorized to add wear and tear on the body, accelerating the physiological processes of aging that may, indeed, affect inflammatory and immunological processes via DNAm mechanisms; thus, these effects are most relevantly studied in blood (Miller et al. 2009, Zannas et al. 2015). For example, in a study assessing the effects of extreme social deprivation, Esposito et al. (2016) reported DNAm findings as well as cell type composition of peripheral blood mononuclear cells as outcomes given the hypothesized importance of inflammatory systems.

In contrast, the majority of studies in psychology assess DNAm in peripheral tissues, with the primary interest being the brain. For instance, many human studies have sought to explore the link between DNAm at the glucocorticoid receptor gene *NR3C1* in peripheral tissue and early life exposures following animal work reporting DNAm changes in brain tissue (Turecki & Meaney 2016, Pan et al. 2014, Weaver et al. 2004). Methylation at this promoter has low variability across individuals, yielding very small observed effects and uncertainty around whether these effects are consistent in brain tissue, although some work has found consistent patterns in a small cohort of hippocampal samples of suicide victims who experienced early childhood abuse (McGowan et al. 2009). Because bioinformatic methods for correction for interindividual differences in cell type (i.e., hippocampal neurons or glia) were not available in this early work, replication with correction is required.

In addition, developmental studies are typically longitudinal, as time ordering is critical to making inferences about potentially enduring effects of early exposures. Much of the earliest DNAm work in development relied on precollected data and incorporated DNAm analysis of collected samples after the fact at later developmental stages (e.g., Essex et al. 2013). As covered above, the best-case scenario for making a causal inference would be to assess DNAm both pre- and post-exposure, to the extent that exposures are temporally discrete. One candidate DNAm study focusing on promoters of the oxytocin receptor gene and brain-derived neurotrophic factor promoters assessed DNAm at these sites in blood pre- and post-exposure to the Trier social stress test (Unternaehrer et al. 2012). The authors found a change in methylation at the oxytocin receptor; however, pre- and post-stress differences in DNAm were no longer significant after adjustment for blood cell counts. This study thus also highlights the importance of accounting for cell composition when attempting to elucidate the causal effects of exposures. As collection of biological samples becomes more commonplace, the assessment of DNAm changes pre- and post-social exposures in longitudinal contexts is an intriguing possibility for future work.

Candidate Gene Studies

Candidate gene studies in psychology focus on DNAm within one gene or a small number of genes of interest, often in peripheral tissues. The candidate approach is applied in several domains of

psychology research but commonly encompasses neurodevelopmental hypotheses specific to the biological effects of one of several popular candidates related to neurotransmitter or neuropeptide function (e.g., *5-HTTLPR*, *OXTR*, *MAOA*). The neuroimaging epigenetic literature, for instance, almost entirely focuses on these candidates, including *NR3C1*, as discussed above (Nikolova & Hariri 2015). However, one study to date has applied whole epigenome analysis with neuroimaging (Chen et al. 2015).

There are a few noteworthy methodological limitations of the current candidate gene literature. First, there has largely been a focus on candidate gene promoters, which, as mentioned above, tend to exhibit minimal variability, as most promoters are typically unmethylated. Second, strategies such as taking an average or a principal component of multiple CpGs in an effort to collapse DNAm measurements into one methylation variable for further analysis are commonplace. This strategy might be problematic, as groups of CpGs could overlap with different types of functional regions, so collapsing a group together risks removing important functional information. Third, with most candidate studies, the absence of genome-wide data and, typically, lack of cell counts or cell sorting mean that DNAm is quantified across more than a single cell type without the ability to correct for cell type proportions. As the major driver of variation in DNAm is cell type (see above), these results could easily be seriously confounded. There are ways around these drawbacks, however; for example, one study targeted a known functional intronic region and grouped CpGs according to spatial proximity to binding sites for analysis, and findings were replicated in a subsample that accounted for cell type proportions (Klengel et al. 2013).

Psychological Disorders

Brain tissue is of primary interest in the manifestation of psychological disorders, and the epigenetics psychiatric literature has the advantage of brain biobanks available for cases and controls with various psychiatric conditions. For instance, an epigenome-wide study of CpG-rich regions in prefrontal cortex tissues obtained from individuals diagnosed with schizophrenia, individuals diagnosed with bipolar disorder, and controls identified epigenetic modifications involved in neuronal development and metabolism (Hannon et al. 2016, Mill et al. 2008). We note that cell type was not controlled for, as is typical of many studies in this area, and, thus, the association could be driven by cell type differences.

Given the potential link between the historical interaction of genetic factors and exposures and DNAm patterns, another possibility is to use DNAm as a predictor or marker of risk for psychiatric disorders. In a recent article applying epigenome-wide analysis to DNAm in peripheral blood in men, early-life stress in the form of separation from families was not found to relate to DNAm (Khulan et al. 2014). However, later psychological follow-ups revealed that epigenetic signatures predicted the onset of depressive symptoms 5–10 years following sample collection. Although cell type was also not accounted for in this study and, thus, it should be interpreted with caution, it does showcase the potential of DNAm patterns and their possible reflection of earlier interplay of genetic and environmental risk as predictors of psychological outcomes.

CONCLUDING REMARKS

Epigenetics offers an exciting avenue for inquiries into the origin and development of psychological health and human disease. The prominent role of DNAm in early cell differentiation and plasticity makes it an intriguing molecular mechanism for the biological embedding of early experiences but, paradoxically, also introduces the major caveat of its variability being strongly driven by genetic sequence and cell type. To move the study of DNAm in psychological and developmental

processes forward, careful attention to these and other idiosyncratic characteristics of DNAm is critical for study design and interpretation. We hope this review will be useful in guiding researchers in incorporating this promising approach into the study of biological underpinnings in psychological science.

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