Putting the 'epi' into epigenetics research in psychiatry

Abdulrahman M El-Sayed,[1,2] Karestan C Koenen,[1] Sandro Galea[1]

[1]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, New York, USA
[2]College of Physicians and Surgeons, Columbia University, New York, New York, USA

Correspondence to
Dr Abdulrahman M El-Sayed, Department of Epidemiology, Mailman School of Public Health, Columbia University, 722 W. 168th Street, R521, New York, NY 10032, USA; ame2145@columbia.edu

Received 27 January 2013
Revised 13 March 2013
Accepted 14 March 2013
Published Online First 9 April 2013

ABSTRACT

During the past two decades, research concerned with the aetiology of psychopathology has generally progressed along two separate paths: investigations that have characterised the roles played by environmental determinants such as childhood adversity in the development of psychopathology, and those that have focused on neurobiological processes involving genetic and intracellular pathways. Epigenetic modifications, functionally relevant changes to gene expression that do not reflect changes in gene sequence, may explain how environmental exposures 'get under the skin' to modify the expression of genes and produce phenotypic variability. The potential of epigenetic research to unify two disparate strands of inquiry has contributed to substantial, and growing, interest in epigenetics in mental health research. However, there are several challenges with which investigators must contend in studies considering the role of epigenetic modifications in psychopathology. These include the development of causal models in study design, considerations about sample size and generalisability, and robust measurement of epigenetic modification. We employ an epidemiological lens to discuss these challenges and to provide recommendations for future studies in this area.

What is already known about this subject

- The literature about the aetiology of mental disorders remains divided between work focused on environmental determinants such as trauma, past experience and neighbourhoods, on one hand, and neurobiological processes involving genetic pathways and intracellular processes, on the other. Evidence that epigenetic modifications follow environmental exposure and may explain differences in gene expression that accompany such exposures has made epigenetic research a natural point of convergence for population health scientists and neurobiologists interested in the aetiology of complex mental diseases. Therefore, epigenetic mechanisms may help bridge the gap in our understanding about the role of environmental and genetic influences on psychopathology.

What this study adds

- Epigenetic mechanisms may help bridge the gap in our understanding about the role of environmental and genetic influences on psychopathology.
- Methodological challenges to the analysis of epigenetic mechanisms in psychopathology include the development of causal models in epigenetic studies, study design considerations regarding sample size and generalisability, and robust measurement of epigenetic modification.
- We employ an epidemiologic lens to dissect these challenges and provide recommendations for future research in this area.

UNDERSTANDING THE AETIOLOGY OF MENTAL DISORDERS

Much of the literature considering the aetiology of mental disorders remains divided between the work of population health scientists who focus on environmental determinants such as trauma, past experience and neighbourhoods, on one hand, and neurobiologists who focus on genetic pathways and intracellular processes on the other. These parallel lines of inquiry have left a gap in our understanding of the aetiology of psychopathology. While the importance of both genes and environments as determinants of psychopathology is inarguable, the extant work that has attempted to understand gene–environment interactions in the production of psychopathology has been largely limited to investigations of statistical interactions from population data rather than work that has explored the mechanisms through which genes and environments may jointly produce pathology.

Little work has attempted to meaningfully unite these two lines of thought, with a few notable exceptions.[1–4] A groundbreaking series of studies by Meaney and colleagues nearly a decade ago suggested the potential import of epigenetic mechanisms for explaining how environmental exposures 'get under the skin'.[3 4] Although these studies were based on animal models, they offered the potential for understanding how exogenous environmental factors shape population health and disease. Evidence that epigenetic modifications follow environmental exposure and may explain differences in gene expression that accompany such exposures has made epigenetic research a natural point of convergence for population health scientists and neurobiologists interested in the aetiology of complex mental diseases. Therefore, epigenetic mechanisms may help bridge the gap in our understanding about the role of environmental and genetic influences on psychopathology and have been greeted with suitable enthusiasm.[5]

To cite: El-Sayed AM, Koenen KC, Galea S. *J Epidemiol Community Health* 2013;**67**:610–616.

EPIGENETICS AND PSYCHOPATHOLOGY

Epigenetic modifications involve those mitotically heritable, reversible alterations in gene expression occurring independently of changes in DNA sequence.[6] While some classical definitions have focused on intergenerational transfer of phenotype independent of DNA sequence, the psychopathology literature has concentrated more on processes mechanising alterations in gene expression through the life course. We therefore focus here on those processes as well. They occur via four principle mechanisms: DNA methylation, alteration of chromatin structure via histone modification, RNA and protein products that alter gene expression, and prion proteins.[6]

DNA methylation is the process by which methyl groups are placed on the fifth position of pyramidine rings of cytosine-guanine (CpG) dinucleotides,[7] which are common in gene promoter regions.[8] The reaction is catalysed by a group of enzymes, known as DNA methyltransferases.[7] CpG methylation at gene promoter regions impedes the binding of transcription factors, and attracts methyl-binding proteins that ultimately compact chromatin and suppress gene expression.[9] Another common epigenetic mechanism involves the alteration of histones, proteins around which DNA is wrapped. Covalent modifications of histones are involved in mediating changes in the structure of chromatin to facilitate or impede access to DNA by transcription factors, and therefore facilitate or impede expression.[10 11] Predictably, epigenetic modification, including CpG methylation and histone modification, is fundamental to cell differentiation and specialisation throughout eukaryotic embryogenesis and development.[12] A third mechanism involves imprinting by non-coding RNAs and/or protein gene products. In this circumstance, these macromolecules act to alter chromatin structure, transcription, RNA splicing, editing and translation—ultimately shaping gene expression.[13] The best-known example of epigenetic alteration of gene expression by RNA is the inactivation of secondary X-chromosomes in mammalian females.[13] Recent research has also identified a fourth epigenetic mechanism. Prions are remarkably stable proteins that have the capacity to alter protein folding by acting as conformational templates. Prions can then act epigenetically by 'sidestepping' nucleic acid metabolism altogether to alter protein conformation and therefore structure and function in the cell.[14] While epigenetic prions have yet to be demonstrated in humans, research has demonstrated that prion proteins may play an important role in yeast evolution.[15]

Epigenetic modification of gene expression remains active beyond the initial phases of development in which these modifications are most well characterised. In fact, changes in gene expression via epigenetic modification have been shown to mediate the relationship between environmental stimuli and physiological—and pathophysiological—change throughout the life course.[16] In this way, epigenetics may unite neurobiology and population health around understanding how exposure to social, environmental and contextual traumas may modify physiological function to produce psychiatric disease in populations.

Early research on epigenetic mechanisms in psychopathology in humans has been promising.[17] Studies have demonstrated epigenetic modification in the aetiology of autism,[18] schizophrenia,[19] bipolar disorder,[19 20] depression,[20 21] anxiety disorders[22] and suicide.[23 24] Other work has explicitly linked epigenetic modifications to functional changes in gene expression.[24] Ernst and colleagues, for instance, studied postmortem brain tissue from suicide completers and matched controls and found significant differences in epigenetic modification at a locus of interest in the frontal cortices of subjects who had completed suicide as compared with controls, and that the frequency of modification at specific sites were associated with downregulation of the gene-product of interest.[24]

However, while there is a growing literature interested in the roles of epigenetic modification in the aetiology of psychopathology in humans, the design and analysis of studies in this area are fraught with considerable epidemiological challenge.[25] This includes the development of causal models in study design; sample considerations, including size and generalisability; and the proper measurement of epigenetic modification. Using epidemiological principles to explore these challenges, we suggest here directions for future research about the role of epigenetic modification in the aetiology of psychopathology in humans.

CHALLENGES IN ANALYSING THE ROLE OF EPIGENETIC CHANGE IN PSYCHOPATHOLOGY

Causal models in epigenetic studies

The first challenge in epigenetic studies of psychopathology is the specification of causal models consistent with hypotheses about how epigenetic modifications may contribute to the production of phenotypes of interest. Causal models are heuristics that formalise our thinking about the nature, directionality and temporality of hypothesised relationships between covariates in population data.[26] In forcing us to formalise our hypotheses prior to analysis, these models help us articulate our research questions in light of current knowledge, as well as the limitations of our data. While causal models are implicit in epigenetic studies, they are rarely formalised. Rather than test formal hypotheses, therefore, about how epigenetic modification may be associated with either environmental exposures and/or outcomes of interest, existing studies have been limited to exploratory analyses that do not adequately address current questions in the field. As a potential guiding heuristic, we propose a directed acyclic graph for a generalised epigenetic pathway by which an environmental exposure may operate via epigenetic modification to produce an outcome of interest, shown in figure 1. The graph demonstrates potential mediation and confounding germane to causal inference in epigenetic studies, as well as the temporal relationships in which these factors must be measured and in which they must be accounted.

The paucity of well developed and articulated causal models in epigenetic studies is endemic in this literature, and has had several consequences. These include: (1) absent or inappropriate specification of environmental exposures antecedent to epigenetic modification, (2) inappropriate adjustment for confounding by environmental or genetic factors associated with both epigenetic modification and outcomes and (3) lack of attention paid to the temporal relationship between epigenetic modification and outcomes.

First, extant studies in the field have not incorporated data about the environmental exposures that are thought to initiate epigenetic change; in a recent review of the literature,[27] only three of 21 studies sought to assess the differential influence of epigenetic modifications on psychiatric outcomes among individuals with a history of a common environmental stressor.[23 28 29] Despite ample population data suggesting that psychopathology is environmentally determined,[30] our studies are not equipped to address dominant hypotheses about the mediating role of epigenetic changes in these relationships because they have not assessed epigenetic modification in relation to environmental exposures known to predict these outcomes. Moreover, it may not be sufficient to measure such exposures at one point in time. For example, it is plausible that cumulative exposure, the

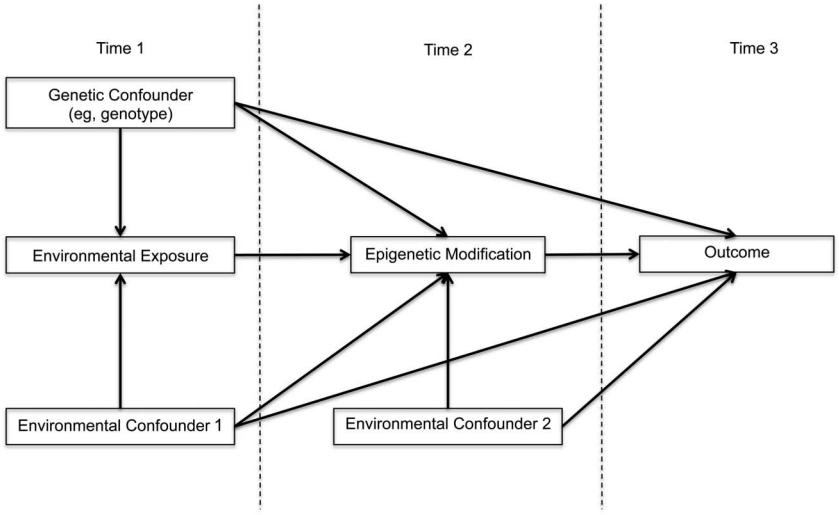

Figure 1 A directed acyclic graph illustrating a general framework for causal models involving epigenetic pathways relating an environmental exposure and an outcome.

most extreme level of exposure at any time point or exposure level during a particularly sensitive point in the life course may be more important in the aetiology of outcomes of interest, and therefore, investigators should take care to operationalise exposures of interest in several different ways.

Second, unmeasured confounders are pervasive in this literature and challenge the validity of existing studies in the area. Confounders are third variables that predict both exposure and outcome but do not mediate the relationship between them.[31 32] In studies about the relation between epigenetic modification and psychopathology it is plausible that environmental factors that both cause epigenetic change and independently cause psychiatric outcomes may confound the relationship in question. For example, studies in animal models have demonstrated that maternal social support during infancy, but also social support from others, including fathers, siblings and extended family members throughout youth, produces epigenetic changes that influence social behaviour in adult life.[33] Extending to observational studies about psychopathology in humans where experimentation is not possible, it is necessary, then, to differentiate the social influences of peer support in later life from the influences of epigenetic modifications resulting from maternal care or child maltreatment on outcomes of interest.

Along with environmental confounders, specific alleles may also confound the relationship between epigenetic modification and outcomes. In this regard, recent research has demonstrated the existence of allele-specific methylation at up to 37% of heterozygous loci.[34 35] In this way, some alleles may be more likely than others to be epigenetically modified, independent of environmental exposures or outcomes. If these alleles are also more likely to occur at loci implicated in the aetiology of diseases in question, independent of methylation status, such as the 's-type' allele of the serotonin transporter locus with respect to depression,[36] failing to adjust for genotype can result in spurious associations between epigenetic regulation and psychopathology.

Third, an irrevocable condition for causal inference is that exposures must precede outcomes in epidemiological studies.[31] Plausible causal models require explicitly articulated temporal relationships among environmental exposures, epigenetic modifications and outcomes of interest. However, to this point, the literature in this area, relegated to cross-sectional studies, has measured epigenetic modification concurrently with outcomes of interest, and in the case of postmortem brain studies, following these outcomes. In this way, it is impossible to disentangle epigenetic modifications that may have resulted from outcomes themselves from those modifications that may have caused them. For example, a recent cross-sectional case-control analysis by Uddin and colleagues contrasted methylation profiles from peripheral blood from a population of adults with a history of depression with those of non-depressed adults.[37] The study suggested differences in methylation profiles in genes involved in brain development and tryptophan metabolism between cases and controls.[37] However, given the cross-sectional design of the study, it is impossible to ascertain the temporality of the relationship between these methylation differences and depression. While it is plausible that these changes are involved in the aetiology of depression, it is also possible that they may be the result of depression itself. In this way, the question of temporality between epigenetic modifications and pathology remains a substantial challenge to understanding the role of epigenetic modification in psychiatric disease.

Sample characteristics in epigenetic studies

A second important challenge to overcome in studies of epigenetic modification in the aetiology of psychopathology is limitation by characteristics of the sample. Samples that are not adequately sized or that are not representative pose particular challenges to study validity. First, samples must be adequately sized to afford the study sufficient power. Studies with small samples, and therefore low power, increase the chances of Type II error, or false negative findings. More insidiously, however, small samples can also increase the chances of Type I error, increasing the likelihood of false positive findings.[38–40] The false discovery rate (FDR), the proportion of significant findings in a field that are actually the product of Type I error, given by equation 1, is a function of the specified Type I error rate (α), the prior probability that a hypothesis is correct, and the study power.

$$\mathrm{FDR} = \frac{\alpha(1-\mathrm{prior})}{\alpha(1-\mathrm{prior}) + \mathrm{power} * \mathrm{prior}} \quad (1)$$

As equation 1 demonstrates, FDR is inversely proportional to the study power, suggesting that as sample size increases, and power increases, the FDR (Type I error across studies) should decline. In this way, sample size is a crucial consideration for statistical validity in these studies.

Small sample sizes are a common problem in molecular epidemiological studies.[41] However, the limitations imposed by small samples size are particularly acute when considering epigenetic

studies because of the inherent potential for confounding by both environmental factors as well as genotypes in these studies. In this way, the requirement of adjusting for these multiple potential confounders increases the number of degrees of freedom used in regression models, imposing a lower bound on tenable sample sizes in epigenetic studies. Moreover, as mentioned above in our discussion of causal models, testing epigenetic hypotheses implies mediation analysis, requiring larger sample sizes than simple exposure–outcome assessments in epidemiological studies.

A second consideration with respect to samples in epigenetic studies is the degree to which they are representative of the population in question, a key concern for both statistical and external validity. With respect to statistical validity, studies that oversample particular populations may allow for a high degree of clustering or shared characteristics between individuals in samples. These shared characteristics may violate the assumption of independence of observations: the fundamental assumption in most regression modelling techniques that the characteristics of individuals that influence the likelihood of developing an outcome are independent from one another.[42 43] In this way, analyses of data with a high degree of clustering may bias study findings. Clustering also compounds the sample size problems discussed above, as it decreases the effective sample size in regression models,[44] forcing investigators to recruit even larger samples to ensure statistical validity. Moreover, samples that are not representative with respect to geography, age distribution, race and/or ethnicity, or baseline health may limit the external validity of findings. Poor generalisability limits our capacity to translate epigenetic findings into meaningful, population level interventions.

Measuring epigenetic modification

A third challenge in epigenetic studies of psychopathology is the appropriate measurement of epigenetic modification. There are here several issues. The first concerns *what* to measure, the second concerns *where* to measure, the third concerns *when* to measure and the fourth *how* to measure epigenetic change with respect to psychopathology.

The question of *what* to measure is crucial. As discussed above, epigenetic modifications involve DNA methylation or alteration of chromatin structure that either facilitates or impedes access to DNA by transcription factors and their associated complexes, ultimately modulating gene expression.[6] However, some studies have only measured epigenetic modification in the form of DNA methylation or histone modification,[27] neglecting to measure gene expression profiles in the form of RNA or protein gene products, assuming rather that epigenetic modification should imply concomitant changes in gene expression. However, this assumption is not always met; in some circumstances, epigenetic changes may not be accompanied by suspected alterations to gene expression.[45] In this way, directly measuring epigenetic modification without also measuring changes in gene expression profiles is inappropriate.

With respect to the question of *where* to measure, studies about epigenetic modification in the aetiology of psychiatric diseases have measured markers of epigenetic modification in several tissues, including peripheral blood cells,[46] other peripheral tissues (such as buccal mucosal cells)[47] and postmortem brain cells.[48] While all nucleated human cells host the full complement of genetic material, different cells may alter gene expression differently to best accomplish their particular function throughout the course of specialisation, activating some genes while silencing others in line with physiological roles.[10 11] Although the pathophysiology of psychiatric disease remains largely unclear, it is known that psychiatric pathology—the cellular changes that mechanise disease phenotypes—is localised somewhere in the brain.[49] For this reason, measuring epigenetic modification in peripheral tissues, like peripheral blood cells or buccal mucosa, without evidence that these peripheral changes are pathognomonic and specific is problematic.

At the same time, however, the measurement of epigenetic changes in postmortem brain tissue may also impose limitations with respect to *when* to measure. First, postmortem brains can only be harvested after death—and therefore after the occurrence of the outcome of interest. Hence, the measurement of the epigenetic modification can only occur after the outcome has already taken place, imposing a necessary limitation on our ability to ascertain temporality of epigenetic exposure prior to outcome, as discussed above. Second, the process of death often involves acidosis secondary to hypoxaemia. Both acidosis and hypoxaemia may contribute to the instability of genetic material,[50–52] which increases the potential for misclassification of epigenetic modification and spurious findings. Third, limiting studies to postmortem brains may introduce a source of selection bias into epigenetic studies because factors associated with psychopathology may predict the cause of death, which in turn is likely to predict the viability of brain tissue. This imposes considerable limitations to internal validity. On a similar note, the time-horizon of epigenetic changes is unclear; it is possible, therefore, that epigenetic changes that may not induce concomitant changes in gene expression may yet influence gene expression at a later point in the life course. In this regard, studies should consider the downstream influences of epigenetic change on gene expression and psychopathology at multiple points in the life course in order to strengthen causal inference.

Last, the question of *how* to measure epigenetic modification remains a challenge for investigators in this area. There are many laboratory protocols for measuring epigenetic change. In the case of DNA methylation alone, for example, there are several available assays. These include methylated DNA immunoprecipitation;[53] bisulfite reaction based DNA sequencing methods, such as methylation-specific PCR and/or bisulfite genomic sequencing PCR;[54 55] Restriction Landmark Genomic Scanning for Methylation techniques;[56] and genome-wide screens, such as CpG island microarrays.[57] More recently, the Reduced Representation Bisulfite Sequencing (RRBS) method and the Illumina HumanMethylation450 beadchip (Illumina 450K) have come into more common use. While the sensitivity and specificity of these approaches relative to one another, to the best of our knowledge, remains unmeasured, one recent study pursued a head-to-head comparison of two very similar bisulfite sequence based assays in human embryonic stem cell replicates and found a nearly 20% difference in methylated CpG islands identified.[58] Despite the more common use of the RRBS and Illumina 450K protocols, there remains no gold standard assay. In this way, differential use of assays may lead to non-differential misclassification bias, ultimately increasing the chances of Type II error in this literature.

Disease-discordant monozygotic twin studies

Investigators have advocated the use of disease-discordant monozygotic twin analyses as a convenient study design for analyses regarding the role of epigenetic modifications in psychopathology,[59] and these studies have been used previously to understand the aetiology of psychopathology.[60 61] For example, in one of the earliest studies using this design, Petronis and colleagues showed differences in methylation patterns of lymphocyte DRD2 in schizophrenia-discordant monozygotic twins.[60]

While disease-discordant monozygotic twin studies pose a unique study design because they control for confounding by genotype as well as shared environment between twins raised together,[62 63] they remain subject to some of the limitations discussed above. First and principally, while in some circumstances, epigenetic-discordance in the setting of disease-discordance may imply epigenetic mechanisms in the aetiology of disease, the relationship between epigenetic change and the outcome of interest may still be confounded by differential environmental exposures acting both to produce the epigenetic modifications of interest but also independently to influence the likelihood of the outcome. In this regard, while investigators have argued that monozygotic twin studies can address 'unknown confounders',[59] these studies only address several known sources of confounding, confounding by genotype and some sources of confounding by shared environment. Moreover, while twins raised together often share environmental exposures at the level of the locality, the household and the family, experiences within those environments may differ, and it may not be appropriate to assume exchangeability across environmental exposures between these twins. This is particularly true with respect to psychopathology, where environmental exposures of interest—such as parental affection or perceived stress—are impossible to aggregate at the family or household level.

Second, the challenge of generalisability persists and may, in fact, be more acute in the setting of monozygotic twin studies. Generalisability is especially challenged in studies that consider the aetiology of psychopathology, as it is plausible that this population may face stressors and protective factors particular to monozygotic twins; these include, for example, differences in neonatal brain structure between twins and singletons.[64]

BRIDGING THE GAP: DIRECTIONS FOR EPIGENETIC RESEARCH IN PSYCHOPATHOLOGY

With the potential to unite population health and neurobiology around a coherent, unified approach to understanding how environmental exposures get under the skin to produce psychiatric phenotypes, epigenetic approaches to psychopathology have tremendous potential. However, as we outline above, realising this potential is contingent upon moving beyond several methodological challenges in the field. Broadly, investigators must be aware of the strengths and weaknesses of the study designs they employ. While designs such as disease-discordant monozygotic twin studies have useful properties that facilitate causal inference, they do not solve all problems with confounding and may not be generalisable to singletons. Care must be taken to interpret findings accordingly.

More specifically, the first methodological challenge to address is the specification and employment of causal models consistent with hypotheses about how epigenetic modifications may contribute to the production of phenotypes of interest. The second challenge is obtaining samples of sufficient size and representativeness to allow for meaningful statistical inference. And the third challenge is answering the questions of where, when, how and what to measure with respect to epigenetic changes in these studies.

We offer here several directions for future work. Addressing the first challenge regarding causal models in epigenetic studies will fall on the shoulders of individual investigators—we would do well to fully articulate causal models that formalise our hypotheses and guide our study designs and analytic plans prior to initiating studies. Furthermore, studies that consider only epigenetic phenomena in relation to outcomes may not be sufficient. In order to truly test our hypotheses about the role of epigenetic phenomena in explaining the mechanisms by which environmental exposures and social experiences shape psychiatric disease risk, our studies will have to include data about those very factors that may operate as antecedent exposures to epigenetic modification or as confounders of the relationship between epigenetic modification and outcomes.

Moreover, future studies will need to consider more deeply the potentially confounding effects of genotype, as it is plausible that there may be confounding by allele variant. Similarly, epigenetic studies should measure epigenetic modification at more than one point in time. In order to establish epigenetic modification as a mediator between environmental exposure and an outcome of interest, this modification must occur after the environmental exposure and prior to the outcome. In this way, future studies should employ prospective cohort designs that allow for regular, periodic assessment of epigenetic modification in tissues of interest to address the temporal relationship among environmental exposures, epigenetic change and psychiatric outcomes.

We also considered the challenge of obtaining samples that are adequate in size and representativeness. As in all studies, the ideal cohorts would be large, diverse and population-representative. Moving away from the convenience samples that have characterised studies in the field, such cohorts would allow for adequate powering of analyses and avoid problems of clustering and interclass correlation. Large, diverse, representative samples minimise problems of Type I and Type II errors that have dogged molecular epidemiological studies,[39] and improve the generalisability of study findings. Moreover, as the extant research has been relegated to small, selected samples, reproducing studies in different contexts and among different populations groups is needed to further generalise our understanding of the role of epigenetic modifications in the aetiology of psychopathology.

We considered the questions of where, when, how and what to measure with respect to epigenetic change in the aetiology of psychopathology. With respect to the question of *what*, it is clear that simply measuring direct epigenetic modification (eg, methylation or histone alteration) is inadequate; such changes do not necessarily imply alteration in gene expression—the presumed mechanism by which they act. In this way, studies in this area should both measure epigenetic change as well as relevant gene expression profiles to assure that epigenetic modification has had the expected effect on cell physiology. Similarly, with respect to the question of *how* to measure epigenetic change, research is needed to produce and validate gold standard measures of epigenetic modification. If and after such gold standards are established, studies in this area would do well to adhere to them in the absence of compelling reasons not to do so. In the interim, however, as is required by many journals in the field, findings should be validated across multiple assays to ensure that they are robust and to minimise misclassification bias.

Addressing the questions of *where* and *when* to measure epigenetic modification is considerably more complex. In the ideal scenario, we would obtain repeated measures of epigenetic modifications from samples from any region of interest in the live brain. However, given the obvious limitations to sampling live brains among the general population, we are limited at this time to either measuring brain tissue after death, or peripheral tissue in live subjects, both of which have important drawbacks, as we described above. There is, therefore, a direct trade-off between measurement of the appropriate *where* and the appropriate *when*.

While, at this point, this trade-off is unavoidable in population studies (save sampling the highly selected population of neurosurgical patients, for whom only a limited sampling of

tissues can be harvested), future work attempting to validate postmortem brain epigenetic markers and/or to understand the relationship between epigenetic modification in the peripheral tissues with that in the brain may be fruitful. Moreover, future work in brain imaging to identify functional magnetic resonance images correlating to gene expression—and ideally epigenetic modification—would allow us a real-time view of the role of epigenetic modification in psychopathology. Access to suitable proxies for epigenetic modification in real-time would also allow for a life course understanding of the role of epigenetic modification in psychiatric disease, which would move us well beyond our current understanding of the role of epigenetic processes in psychopathology.

There remain a number of important questions regarding the role of epigenetic modifications in the aetiology of psychopathology. While most of this work has focused on epigenetic modifications and subsequent pathology in the individual, an important direction for future work will be the study of epigenetically-mediated intergenerational transfer of psychopathology. In this respect, promising research in animal models has demonstrated, for example, that opioid and cannabinoid exposure in maternal adolescence may influence addiction-like and anxiety-like behaviour in subsequent generations.[65–67] Other work has demonstrated how epigenetic alterations in the sperm of cocaine-sired fathers may influence cocaine-resistance in male progeny.[68] These studies and others represent important departures for human studies regarding the role of epigenetics in psychopathology. Methodologically robust studies concerned with epigenetics in human populations stand to make an important contribution to our understanding of the aetiology of mental diseases by uniting neurobiology and population health science.

Acknowledgements Funding sources include NIH grants DA022720, DA022720-S1, MH092526, MH088283, MH078928 and MH 093612 5P51RR000165.

Contributors AME-S conceived of and drafted the manuscript. KCK and SG consulted on technical aspects of the manuscript and critically reviewed the manuscript for intellectual content.

Funding This work was supported by National Institutes of Health grant number DA022720, DA022720-S1, MH092526, MH088283, MH078928 and MH 093612 5P51RR000165.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Caspi A, Hariri AR, Holmes A, *et al*. Genetic sensitivity to the environment: The case of the serotonin transporter gene and its implications for studying complex diseases and traits. *Am J Psychiatry* 2010;167:509–27.
2 Ressler KJ, Mercer KG, Bradley B, *et al*. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature* 2011;470:492–97.
3 Weaver ICG, Cervoni N, Champagne FA, *et al*. Epigenetic programming by maternal behavior. *Nat Neurosci* 2004;7:847–54.
4 Zhang TY, Meaney MJ. Epigenetics and the Environmental regulation of the genome and its function. *Ann Rev Psychol* 2010;61:439–66.
5 Carey B. Genes as mirrors of life experiences. *New York Times 2010*. 11 September 2010; D7.
6 Henikoff S, Matzke MA. Exploring and explaining epigenetic effects. *Trends Genet* 1997;13:293–5.
7 Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci* 2006;31:89–97.
8 Bird AP. CpG-rich islands and the function of DNA methylation. *Nature* 1986;31:209–13.
9 Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 2003;33:245–54.
10 Spencer VA, Davie JR. Role of covalent modifications of histones in regulating gene expression. *Gene* 1999l;240:1–12.
11 Berger SL. The complex language of chromatin regulation during transcription. *Nature* 2007;447:407–12.
12 Li E. Chromatin modification and epigenetic reprogramming in mammalian development. *Nat Rev Genet* 2002;3:662–73.
13 Mattick JS, Makunin IV. Non-coding RNA. *Hum Molec Genet* 2006;15:R17–29.
14 Halfmann R, Lundquist S. Epigenetics in the extreme: prions and the inheritance of environmentally acquired traits. *Science* 2012;330:629–32.
15 Patel BK, Gavin-Smyth J, Liebman SW. The yeast global transcriptional co-repressor protein Cyc8 can propagate as a prion. *Nature Cell Biology* 2009;11:344–52.
16 Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. *Nat Rev Genet* 2007;8:253–62.
17 Tsankova N, Renthal W, Kumar A, *et al*. Epigenetic regulation in psychiatric disorders. *Nat Rev Neurosci* 2007;8:355–67.
18 Miyake K, Hirasawa T, Koide T, *et al*. Epigenetics in autism and other neurodevelopmental disorders. *Adv Exp Med Biol* 2012;724:91–8.
19 Labrie V, Pai S, Petronis A. Epigenetics of major psychosis: progress, problems and perspectives. *Trends Genet* 2012;28:427–35.
20 Cruceanu C, Alda M, Nagy C, *et al*. H3K4 tri-methylation in synapsin genes leads to different expression patterns in bipolar disorder and major depression. *Int J Neuropsychopharmacol* 2012;16:1–11.
21 Fuchikami M, Morinubu S, Segawa M, *et al*. DNA methylation profiles of the brain-derived neurotrophic factor (BDNF) gene as a potent diagnostic biomarker in major depression. *PLoS One* 2011;6:e23881.
22 Esler M, Alvarenga M, Pier C, *et al*. The neuronal noradrenaline transporter, anxiety and cardiovascular disease. *J Psychopharmacol* 2006;20:60–6.
23 McGowan PO, Sasaki A, Huang TC, *et al*. Promoter-wide hypermethylation of the ribosomal RNA gene promoter in the suicide brain. *PloS One* 2008;3:e2085.
24 Ernst C, Deleva V, Deng X, *et al*. Alternative splicing, methylation state, and expression profile of tropomyosin-related kinase B in the frontal cortex of suicide completers. *Arch Gen Psychiatry* 2009;66:22–32.
25 Heijmans BT, Mill J. Commentary: The seven plagues of epigenetic epidemiology. *Int J Epidemiol* 2012;41:74–8.
26 Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology* 1999;10:37–48.
27 El-Sayed AM, Haloossim MR, Galea S, *et al*. Epigenetic modifications associated with suicide and common mood and Anxiety Disorders: a systematic review of the literature. *Biol Mood Anxiety Disord* 2012;2:10.
28 McGowan PO, Sasaki A, D'Alessio AC, *et al*. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci* 2009;12:342–8.
29 Klempan TA, Ernst C, Deleva V, *et al*. Characterization of QKI gene expression, genetics, and epigenetics in suicide victims with major depressive disorder. *Biol Psychiatry* 2009;66:824–31.
30 Kim D. Blues from the neighborhood? Neighborhood characteristics and depression. *Epidemiol Rev* 2008;30:101–17.
31 Rothman KJ. Causation and causal inference in epidemiology. *Am J Public Health* 2005;95:S144–50.
32 Greenland S. Confounding in health research. *Annu Rev Public Health* 2001;22:189–212.
33 Cushing BS, Kramer KM. Mechanisms underlying epigenetic effects of early social experience: the role of neuropeptides and steroids. *Neurosci Biobehav Rev* 2005;29:1089–105.
34 Shoemaker R, Deng J, Wang W, *et al*. Allele-specific methylation is prevalent and is contributed by CpG-SNPs in the human genome. *Genome Res* 2010;20: 883–9.
35 Tycko B. Allele-specific DNA methylation: beyond imprinting. *Hum Mol Genet* 2010;19(R2):R210–20.
36 Caspi A, Sugden K, Moffitt TE, *et al*. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 2003; 301:386–9.
37 Uddin M, Koenen KC, Aiello AE, *et al*. Epigenetic and inflammatory marker profiles associated with depression in a community-based epidemiologic sample. *Psychol Med* 2011;41:997–1007.
38 Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Statist Soc B* 1995;57:289–300.
39 Duncan LE, Keller MC. A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. *Am J Psychiatry* 2011;168:1041–9.
40 Wacholder S, Chanock S, Garcia-Closas M, *et al*. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J Natl Cancer Inst* 2004;96:434–42.
41 Burton PR, Hansell AL, Fortier I, *et al*. Size matters: just how big is BIG?: quantifying realistic sample size requirements for human genome epidemiology. *Int J Epidemiol* 2009;38:263–73.
42 Kenny DA, Judd CM. Consequences of violating the independence assumption in analysis of variance. *Psychol Bull* 1986;26:422–31.
43 Cohen J. *Applied multiple regression/correlation analysis for the behavioral sciences*. Mahway, NJ: Laurence Erlbaum Associates, 2003.
44 Burton P, Gurrin L, Sly P. Extending the simple linear regression model to account for correlated responses: An Introduction to generalized estimating equations and multi-level modeling. *Stat Med* 1998;17:1261–91.

45 Philibert RA, Sandhu H, Hollenbeck N, *et al*. The relationship of 5HTT (SLC6A4) methylation and genotype on mRNA expression and liability to major depression and alcohol dependence in subjects from the Iowa adoption studies. *Am J Med Genet B Neuropsychiatr Genet* 2008;147B:543–9.
46 Luca VD, Viggiano E, Dhoot R, *et al*. Methylation and QTDT analysis of the 5-HT2A receptor 102C allele: Analysis of suicidality in major psychosis. *J Psychiatr Res* 2009;43:532–7.
47 Olsson CA, Foley DL, Parkinson-Bates M, *et al*. Prospects for epigenetic research within cohort studies of psychological disorder: a pilot investigation of a peripheral cell marker of epigenetic risk for depression. *Biol Psychol* 2010;832: 159–65.
48 Alt SR, Turner JD, Klok MD, *et al*. Differential expression of glucocorticoid receptor transcripts in major depressive disorder is not epigenetically programmed. *Psychoneuroendocrinology* 2010;35:544–56.
49 Andreason NC. Linking mind and brain in the study of mental illnesses: a project for a scientific psychopathology. *Science* 1997;275:1586–93.
50 Vawter MP, Tomita H, Meng F, *et al*. Mitochondrial-related gene expression changes are sensitive to agonal-pH state: implications for brain disorders. *Mol Psychiatry* 2009;11:663–79.
51 Tomita H, Vawter MP, Walsh DM, *et al*. Effect of agonal and postmortem factors on gene expression profile: quality control in microarray analyses of postmortem human brain. *Biol Psychiatry* 2004;55:346–52.
52 Ernst C, McGowan PO, Deleva V, *et al*. The effects of pH on DNA methylation state: in vitro and post-mortem brain studies. *J Neurosci Methods* 2008;174:123–5.
53 Mohn F, Weber M, Shcubeler D, *et al*. Methylated DNA immunoprecipitation (MeDIP). *Methods Mol Biol* 2009;507:55–64.
54 Herman JG, Graff JR, Myöhänen S, *et al*. Methylation-specific PCR: A novel PCR assay for methylation status of CpG islands. *Proc Natl Acad Sci USA* 2006;93:9821–6.
55 Darst RP, Pardo CE, Ai L, *et al*. Bisulfite sequencing of DNA. *Curr Protoc Mol Biol* Jul 2010; Chapter 7:Unit 7.9.1–17.
56 Akama TO, Okazaki Y, Ito M, *et al*. Restriction landmark genomic scanning (RLGS-M)-based genome-wide scanning of mouse liver tumors for alterations in DNA methylation status. *Cancer Res* 1997;57:3294–9.
57 Yan PS, Chen C, Shi H, *et al*. Applications of CpG island microarrays for high-throughput analysis of DNA methylation. *J Nutr* 2002;132:2430S–4S.
58 Harris RA, Wang T, Coarfa C, *et al*. Comparison of sequencing-based methods to profile DNA methylation and identification of monoallelic epigenetic modifications. *Nat Biotechnol* 2010;28:1097–105.
59 Bell JT, Spector TD. A twin approach to unraveling epigenetics. *Trends Genet* 2011;27:116–25.
60 Petronis A, Gottesman II, Kan P, *et al*. Monozygotic twins exhibit numerous epigenetic differences: clues to twin discordance? *Schophr Bull* 2003;29:169–78.
61 Kuratomi G, Iwamoto K, Bundo M, *et al*. Aberrant DNA methylation associated with bipolar disorder identified from discordant monozygotic twins. *Mol Psychiatry* 2008;13:429–41.
62 Koenig LB, Jacob T, Haber JR, *et al*. Testing the equal environments assumption in the Children of Twins design. *Behav Genet.* 2010;40:533–41.
63 Mitchell KS, Mazzeo SE, Bulik CM, *et al*. An investigation of a measure of twins' equal environments. *Twin Res Hum Genet* 2007;10:840–7.
64 Knickmeyer RC, Kang C, Woolson S, *et al*. Twin-singleton differences in neonatal brain structure. *Twin Res Hum Genet* 2011;14:268–76.
65 Byrnes JJ, Johnson NL, Carini LM, *et al*. Multigenerational effects of adolescent morphine exposure on dopamine D2 receptor function. *Psychopharmacology* 2013 [Epub ahead of print].
66 Johnson NL, Carini L, Schenk ME, *et al*. Adolescent opiate exposure in the female rat induces subtle alterations in maternal care and transgenerational effects on play behavior. *Front Psychiatry* 2011;2:29.
67 Byrnes JJ, Babb JA, Scanlan VF, *et al*. Adolescent opioid exposure in female rats: transgenerational effects on morphine analgesia and anxiety-like behavior in adult offspring. *Behav Brain Res* 2011;218:200–5.
68 Vassoler FM, White SL, Schmidt HD, *et al*. Epigeneticinheritance of a cocaine-resistance phenotype. *Nat Neurosci* 2013;16:42–7.