Epigenetics of addiction: Current knowledge, challenges and future directions

SELF-ARCHIVING VERSION

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Abstract

Addiction to psychoactive substances is a debilitating condition underpinned by the interplay of genetic and environmental factors. At present, a key challenge for research is to delineate how, at a molecular level, these influences become 'biologically embedded', contributing to the onset and persistence of addictive behaviours. Recently, epigenetic processes that regulate gene expression have emerged as a potential mechanism of interest. In this commentary, we discuss the relevance of epigenetics to addiction research, starting with the current state of knowledge, what challenges we have yet to overcome, and what the future may hold in terms of research methodology and translational potential.

What we have learned so far

The 'epigenome' refers to a collection of processes that influence when and where genes are expressed, without changing the DNA sequence itself. One of these processes, DNA methylation (DNAm), has received much attention. DNAm refers to the addition of a methyl group to DNA base pairs – primarily the cytosine base in C-G dinucleotides – which has been observed to repress transcription, resulting in decreased gene expression (Jones, 2012). Studies have found that DNAm: (i) is influenced by genetic architecture (e.g. cis-SNP effects; McRae et al., 2014); (ii) is sensitive to pre- and postnatal environmental exposures (e.g. nutrition, toxins, stress; Kofink et al., 2013); (iii) plays an essential role in normative development (e.g. cellular differentiation, ageing; Smith and Meissner, 2013); and (iv) disrupted patterns are associated with altered biological processes and the emergence of disease states (Klengel et al., 2014). Consequently, interest in the potential role of DNAm in addiction is fast increasing.

Much of what we currently know about DNAm and addiction has come from animal studies, which enable the experimental manipulation of important factors such as the type, extent and timing of substance exposure. These have begun to shed light into the complex, reciprocal and developmentally-moderated relationship between substance use/exposure, DNAm and addiction. For example, exposure to substances (as early as preconception) has been shown to alter DNAm patterns in the brain (e.g. Govorko et al., 2012). In turn, these can mediate gene activation in regions involved in reward processing(e.g. hypothalamus) and memory consolidation (e.g. hippocampus), driving long-term neuroadaptations that underlie the onset and persistence of addiction (Gangisetty et al., 2014; Nestler, 2014). Animal studies have also provided some tentative evidence for intergenerational transmission of DNAm patterns implicated in addiction risk (e.g. Finegersh and Homanics, 2014) as well as normalization of drug-induced DNAm changes by chemical intervention (e.g. Bekdash et al., 2013). In humans, studies have also supported a link between DNAm and addiction, reporting methylomic differences (e.g. in neurotransmitter genes) between substance users and drug-free controls across a number of tissue types and substances (Cecil et al., 2015; Harlaar and Hutchison, 2013).

What our biggest challenges are and how they may be addressed

Despite these promising findings, research on DNAm and addiction currently faces a number of challenges that limit the conclusions that can be drawn.

1. Limited knowledge of the epigenome

Commonly used platforms only capture a small percentage of the methylome (e.g. Illumina 450k, <2%) and typically focus on CpG-rich 'islands' near promoter regions – as such many regions of potential relevance to addiction remain largely inaccessible (Non and Thayer, 2015). To complicate matters (and in contrast to the genome), DNAm has been shown to vary over time and across multiple factors, including age, tissue and cell-type (Liang and Cookson, 2014). This is especially relevant for addiction – a brain-based disorder, which in human epigenetic studies is either examined in vivo via peripheral tissues (e.g. blood, saliva) or in post-mortem neural tissue, making it difficult to infer epigenetic changes in live brain tissue.

The way forward. Rapid technological advances, such as the development of whole-genome bisulfite sequencing (WGBS), will make it increasingly possible to obtain a more complete picture of DNAm, covering regions relevant to addiction in greater depth. Moreover, the compilation of reference datasets will be crucial for establishing a 'normative' benchmark of DNAm, against which to compare addiction-related epigenetic findings (Shakya et al., 2012). In particular, sampling of multiple tissues over time will make it possible to quantify peripheral-CNS-tissue variability (e.g. Walton et al., 2015) and to establish why certain substance-induced DNAm signatures remain stable while others change over time. Strategies for big data integration will also help to establish the functional significance of addiction-related DNAm changes at different biological levels (e.g transcriptomic, metabolomic, neural; Gomez-Cabrero et al., 2014).

2. Issues with research methodology

DNAm data is multifactorial, high-dimensional and inter-correlated, raising questions about how best it should be analysed (Almouzni et al., 2014). So far, studies on DNAm and addiction have varied widely in methodology (e.g. genomic coverage, quality control, sample size, covariates, analysis, significance threshold) as well as the choice of phenotype (e.g. type of substance, severity of use, clinical features, diagnostic criteria), limiting comparability of findings. Notably, addiction studies using candidate gene vs hypothesis-free, epigenome-wide analyses have generally produced inconsistent results (Cecil, et al., 2015).

The way forward. Guidelines for best practice are continuously being fine-tuned, and the increased availability of standardized pipelines will help maximise convergence across studies (Morris and Beck, 2015). Furthermore, the development of data reduction strategies that draw on the interrelatedness of DNAm data (e.g. network/regional analyses) will help alleviate the burden of multiple testing and move beyond single-site analyses (Rotival and Petretto, 2014; Hass et al., 2015). Replication of findings (e.g. via independent samples/techniques) will also become increasingly important in weeding out false-positives, as was the case for genetic studies. The availability of methylomic data in relation to different drug classes will make it possible to distinguish substance-specific markers from markers that are 'shared' across multiple substances, which may reflect a general liability to addiction. Future work will also be needed to establish how methylomic signatures may vary depending on the phenotype of interest (e.g. chronic vs acute substance exposure; substance use vs abuse vs addiction).

3. Difficulties in establishing causal pathways

Most studies on DNAm and addiction have employed a cross-sectional, case-control design. This is problematic because, unlike the genome, DNAm is sensitive to *both* genetic and environmental factors, raising issues of reverse causation. In other words, it is difficult to establish whether identified DNAm differences are a *predisposing* factor for addiction and/or a *consequence* of long-term substance use. Even when studies have been prospective, DNAm has typically been examined at a single time point, precluding the possibility of examining how substance exposure and DNAm interrelate over time to influence addiction risk.

The way forward. Causal inference may be strengthened by capitalising on cross-species designs, using findings from experimental/mechanistic animal models to inform the investigation of DNAm markers in humans. Studies will also need to better quantify the relative contribution of genetic and environmental factors on DNAm (e.g. via twin, GCTA and GxE analyses; Klengel and Binder, 2015; Trzaskowski and Plomin, 2015) and employ prospective designs to examine whether DNAm patterns *predict* substance use liability, as well as addiction risk. Specifically, this will require the use of longitudinal designs that make it possible to compare pre- vs post-exposure methylomic signatures during adolescence, a key period of vulnerability for the development of substance use disorders (Crews et al., 2007). Collecting repeated-measures data on substance exposure, DNAm and addiction status will also enable to test mediation hypotheses (e.g. via structural equation modelling; Cecil et al.,

2014), while the use of advanced inference methods (e.g. Two-Step Mendelian randomization; Relton and Davey Smith, 2012) will make it possible to use genetic instruments in order to examine causal pathways.

What the future might hold: Implications and translational potential

Epigenetics has been heralded as a key 'missing link' in the aetiology of complex disorders, including addiction. However, as we gain an appreciation of the challenges facing epigenetic research, we must be mindful to manage expectations. Bearing this in mind, there are a number of ways in which epigenetic research may in future contribute to our understanding, prevention and treatment of addiction. In the first place, findings may refine existing models of how risk factors for addiction become biologically embedded. Longitudinal modelling of environmental and epigenetic data may also be used to pinpoint specific windows of biological vulnerability (e.g. prenatal period, adolescence) that may benefit most from preventive action. On the longer term, epigenetic variation in specific genes may be used as biomarkers for substance exposure, addiction risk, and response to treatment. Chemical normalization of aberrant DNAm patterns examined in animal studies may also be extended to humans. Ultimately, this knowledge may inform the development of novel strategies for treating addiction, paving the way for personalised intervention.

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