

Epigenetics in Mental Illness

ABSTRACT

Mental illnesses are complex and multifactorial. Nature (genetic factors) is important in their etiology but nurture (environmental factors) via epigenetic mechanisms is being found to be an additional etiological player. Indeed, studies among identical twins show high rates of discordance, especially for stress syndromes and depression. Specifically, aberrant epigenetic regulation is being found to underlie psychiatric disorders. While epigenetic studies of mental illnesses are relatively still in their infancy, further translational research will surely reveal new insights into their pathophysiology, which in turn will help with the discovery of new targets for treatments, biomarkers, patient stratifications and more 'personalized' treatment.

Keywords: DNA methylation, histone modifications, epigenetic biomarkers, epimutations, theranostics

INTRODUCTION

Historically, the word 'epigenetic' was used to describe the phenomenon of cell identity.¹ Our bodies exhibit a great diversity in cell identity, with over 200 different cell types all depending on one genome. This variation is regulated by a system of biochemical alterations of DNA and histone proteins, which give DNA its structure. Together, these modifications are termed the epigenome.

Amongst the most studied epigenetic modifications are DNA methylation, histone modifications and RNA epigenetics. Epigenetic modifications have importance in gene transcription, but they do not actually modify the coding sequence of the gene itself. While these alterations are heritable, there is also the possibility of

reversing them, with DNA methylation being the most stable and histone modifications being more plastic. This fact has enabled the prospect of epigenetic therapy. Indeed, epimutations have been targeted for new drug innovations, and "turning back on" silenced genes represents a prospective advancement in treatment.

Genetic factors (nature) underlie the etiology of most mental disorders. But studying identical twins is showing that there are high rates of discordance, especially when it comes to depression and stress-related syndromes.² This implies other etiological mechanisms possibly involving nurture. And since epigenetics is a layer between nature and nurture, this has spurred research into epigenetics of mental illness.

Indeed, early studies of epigenetics in mental diseases are aiming to discover how environmental factors affect the epigenetic processes in brain regions. These will hopefully shed light on the pathophysiology of mental diseases, which when integrated into the clinical setting, will in turn guide new targets for treatment, stratify patients for specific personalized treatment, and diagnosis.

INVESTIGATIONS

The pathophysiological mechanisms of mental illnesses remain relatively limited as several barriers still exist. Specifically, mental illness is the result of aberrations in the functions of the brain, which is the organ in the human body that is least understood. Moreover, the genetic studies, which in themselves are also limited, imply that mental illness is complex and polygenic with many genetic variants. Many of these variants are associated with the non-coding regions of the genome.³ Model systems are scarce, as is diseased tissue and cell types that can be studied, as will be discussed below.

Nevertheless, in order to gain molecular mechanistic insights, researches are using the following.

Peripheral Blood

Peripheral blood samples are utilised to study epigenetic signatures in neuropsychiatric disorders. For example, Numata et al.⁴ used peripheral blood samples to study the DNA methylation state of the NR3C1 gene. They found a hypomethylation signature in depressed patients. The gene encodes for a glucocorticoid receptor. From post-mortem studies, under expression of this receptor in the hippocampus is linked to depression and suicide.

In another study using peripheral blood samples, Maffioletti et al.⁵ found aberrant expressions of five microRNAs in the 20 subjects in the depressed arm of the analysis. They suggested that these microRNAs form part of the pathophysiological mechanism in depression. Similarly, another study on microRNA from mononuclear cells using peripheral blood by Fan et al.⁶ found five other microRNAs which were up-regulated in patients with depression.

Enatescu et al.⁷ analysed changes in plasma microRNA profiles of patients with major depression. They found that after 12 weeks of treatment, some were over-expressed while others were under-expressed. They propose that such studies have the potential to identify and validate microRNAs as biomarkers in depression; also, they can offer new targets for treatment by using anti-microRNAs or microRNAs mimics.

Brain Tissue

Biopsies of patient's brain tissue are invasive and provide little material to work with. Moreover, neurons that are post-mitotic cannot be expanded in vitro. Post-mortem brain samples have their own disadvantages as well. For example, they represent late stages of the mental illness and so do not provide insight of the epigenetic aberrations present in early life. Besides end-stage brain samples will not show the true natural picture of disease progression as patients would have received pharmacological treatments, which definitely leave their 'noise' marks. On top of these are effects from fixation methods and storage of the samples.

Despite the above, post-mortem human brain samples have been used in several studies. One such study is that done by Lopez et al.⁸ which showed that miR-1202 is expressed differentially in patients with major depressive disorder. This micro-RNA regulates the gene that codes for glutamate metabotropic receptor-4 (GRM4). From their results, they propose that this micro-RNA forms part of the pathophysiology of depression, can predict response to anti-depressant treatment, and is a potential therapeutic target.

Animal Models

Peripheral blood or post-mortem brain tissues have many variables when they are used to study the pathophysiological mechanisms of mental disorders.

Amongst them are sex, race, age, living conditions, medication, and time of collection after death. These can greatly impact the results. Using animal models provide a more controlled approach.⁹ Other advantages of using animal models are that one can study the effects of pharmacological agents and gene editing, which are not possible when using human patients.

Using mice models, Baubry et al.¹⁰ and O'Connor et al.¹¹ showed that micro-RNAs can be potential markers of response to anti-depressant treatments. Also, Grayson et al.¹² studied the DNA methylation profiles in adult offsprings of two mouse models of schizophrenia, i) the 'prenatal restraint stress model' and ii) the 'chronic methionine mouse model'. The adult offsprings showed behavioural and epigenetic and other biochemical deficits. These and similar studies on animal models are providing solid evidence that early-life adversity and other environmental factors can mediate long-lasting epigenetic modifications in the brain, which are conducive to mental ill-health.

Cellular Models

Cellular reprogramming is offering another platform to study neuropsychiatric disorders. In their review, Seshadri et al.¹³ state that induced pluripotent stem cells (iPSCs) and their neural derivatives are being used to understand schizophrenia. Indeed, the results, namely relating to abnormalities in neurotransmission, neurodevelopment, and oxidative phosphorylation, support those arising from other study designs. Viswanath et al.¹⁴ similarly reviewed cellular models to study bipolar disorders.

One great advantage of the above traditional iPSC technology, when used to model human diseases, is that the induced cells have the whole genome of the donor and this makes them fit to dissect diseases caused by genetic errors. This platform is becoming more valuable when combined with CRISPR/Cas9 gene editing and genome-wide association studies. However, when it comes to studying epigenetics it faces a problem, namely that during the process of reprogramming, the epigenetic memory is erased (epigenetic erasure).¹⁵ Thus, mental illnesses that are epigenetically modified by environmental factors need to be studied by a sister technology called 'transdifferentiation' to generate functional-induced neurons (iNs).¹⁶ Transdifferentiated cells seem to maintain the original epigenetic landscape.^{17,18} However, such studies are still in their infancy.

SCHIZOPHRENIA

Studies are revealing that epigenetic modifications are important in the pathophysiological mechanisms of schizophrenia. Here, the main epigenetic mechanisms mostly studied on post-mortem brain tissue and bio-fluids are DNA methylation, histone modifications, and non-coding miRNAs. Amongst the genes that are being

found to be affected by epigenetic aberrations include those that regulate immune function, neurotransmission and neurodevelopment.¹⁹ It is proposed that some of these epigenetic aberrations are induced through environmental factors which affect brain functions in a patient's life but may also be transmitted across generations (the trans-generational epigenetic inheritance effect).

In the study done by Murata et al.²⁰ betaine levels in peripheral blood samples were found to be low especially in first-episode schizophrenia patients but also in some cases of chronic schizophrenia. Betaine is a methyl-donor and its under-expression was associated with genome wide hypomethylation. The authors propose that the genome-wide hypomethylation from decreased betaine expression might be a puzzle part of the pathophysiology of schizophrenia.

Another puzzle part might be the role of NR3C1 gene which is a glucocorticoid receptor gene. The gene is a key contributor in the regulation of the hypothalamic-pituitary-adrenal axis. Liu et al.²¹ found epigenetic aberrations, specifically DNA hypermethylation of the promoter of this gene. This correlates with other similar findings mentioned below associated with depression, implicating that these mental illnesses might have common biological networks.

There are many other research studies on altered DNA methylation, but also on histone modifications of schizophrenic risk genes. Such studies are in hyperdrive especially those involving the common two histone modifications linked with the active promoters and enhancers of schizophrenic risk genes, specifically H3-trimethyl-Lys4 (H3K4me3) and H3-acetyl-Lys27 (H3K27ac). Amongst the schizophrenic risk genes one finds - RELN, GAD1 and CACNA1C.

MicroRNAs are emerging as important regulators in post-transcriptional gene expression in various human diseases. MicroRNAs have been established as important players in the development of the brain and its neuroplasticity and their aberration are involved in neuropsychiatric diseases including schizophrenia. The study by Santarelli et al.²² supports the fact that miRNA aberrations are important players in the complex pathophysiological mechanisms of schizophrenia. The authors analysed gene expression in the post-mortem prefrontal dorsolateral cortex. They integrated this gene expression analysis with miRNA expression profiles and found an important gene-miRNA interaction biological network. Specifically they identified aberrations in miR-92a, miR-495, and miR-134, which are important in neurodevelopment and oligodendrocyte functions. The dysregulation of miRNAs have also been detected in peripheral blood mononuclear cells (PBMCs) in schizophrenia. Further research will surely exploit this as a potential of miRNA biomarkers in schizophrenia. Translational research will also exploit and harness

miRNA aberrations and their biological networks as new therapeutic targets.

DEPRESSION

Only about 40% of depression is heritable and this points to factors which are not genetic in nature, like stressful life events, especially those that occur in early life. Research on animal models have shown that early-life adversity affects gene expression involving epigenetic mechanisms.²³⁻²⁵

Prenatal stress has epigenetic repercussions on the brain. Jensen Peña et al.²⁶ and O'Donnell et al.²⁷ showed that maternal stress is associated with decreased expression of 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) from the placenta. Normally 11 β -HSD2 shields the foetus from glucocorticoids coming from the mother. The suppression of 11 β -HSD2 is associated with DNA hypermethylation in the promoter of its gene and thus the protection is absent, rendering the offspring more vulnerable - to stress and depression.

In another animal study, Mueller and Bale²⁸ identified DNA hypermethylation of the NGF1-A binding region of the glucocorticoid receptor (GR; Nr3c1) in the hypothalamus of offspring of females that were stressed early in the prenatal period. A similar finding of increased DNA methylation at this site was shown in cord blood taken from infants born to human mothers who, during their pregnancy, were depressed or physically abused.²⁹⁻³¹

In keeping with the above, research has shown that maternal separation and maternal maltreatment also induce epigenetic changes in the brain of animal offspring.^{32,33} Some of these epigenetic changes are lasting but others can be partially reversed pharmacologically.^{34,35} Epigenetic aberration signatures have also been shown in suicide completers. For example, Labont  t et al.^{36,37} found aberrant promoter DNA methylation in several genes in the hippocampus of suicide completers; however, other aberrant epigenetic mechanisms, like miRNA and histone modifications, are also involved in suicidal behaviour.³⁸

Epigenetic studies have also been carried out to investigate epigenetic signatures following antidepressant treatment. Melas et al.³⁹ used a genetic rodent model of depression, specifically the 'Flinders Sensitive Line (FSL) one'. They looked into epigenetic changes with regards to the P11 gene in the frontal cortex of the brain. P11 has been implicated in the pathophysiological mechanisms of depression in humans and rodents alike. They found DNA hypermethylation in the promoter of P11 gene, leading to its under-expression. Importantly they also found that giving escitalopram reversed this hypermethylated profile into a hypomethylated one with re-expression of the P11 gene. Treatment with escitalopram also leads to decreased Dnmt1 and Dnmt3a. The latter are two

DNA methyltransferases that, when over-expressed, are responsible for the hypermethylation pattern of the P11 gene in the adult forebrain neurons.

Despite the availability of various anti-depressant treatments, depressed patients may not show good response, even after several different medications have been tried. This is why specific research in epigenomics⁴⁰ (together with other -omic studies) is focusing on identifying epigenetic biomarkers that could predict the optimal treatment for particular subtypes of depressed patients.

CONCLUSION

Harnessing the knowledge that is forthcoming from the study of epigenetics in mental illnesses will definitely aid in their theranostics. Prevention-wise, epidrugs and other conventional therapies (like psychotherapy) have the potential to reverse the epigenetic alterations associated with environmentally-induced mental illnesses and help in preventing their transmission to future generations.

REFERENCES

- Slack JM. Conrad Hal Waddington: the last Renaissance biologist? *Nature Reviews Genetics*. 2002;3(11):889-95.
- Nestler EJ, Peña CJ, Kundakovic M, et al. Epigenetic Basis of Mental Illness. *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry*. 2016;22(5):447-63.
- Gratten J, Wray NR, Keller MC, et al. Large-scale genomics unveils the genetic architecture of psychiatric disorders. *Nat Neurosci*. 2014;17(6):782-90.
- Numata S, Ishii K, Tajima A, et al. Blood diagnostic biomarkers for major depressive disorder using multiplex DNA methylation profiles: discovery and validation. *Epigenetics*. 2015;10(2):135-41.
- Maffioletti E, Cattaneo A, Rosso G, et al. Peripheral whole blood microRNA alterations in major depression and bipolar disorder. *Journal of Affective Disorders*. 2016;200:250-8.
- Fan HM, Sun XY, Guo W, et al. Differential expression of microRNA in peripheral blood mononuclear cells as specific biomarker for major depressive disorder patients. *Journal of Psychiatric Research*. 2014;59:45-52.
- Enatescu VR, Papava I, Enatescu I, et al. Circulating Plasma Micro RNAs in Patients with Major Depressive Disorder Treated with Antidepressants: A Pilot Study. *Psychiatry Investigation*. 2016;13(5):549-57.
- Lopez JP, Lim R, Cruceanu C, et al. miR-1202 is a primate-specific and brain-enriched microRNA involved in major depression and antidepressant treatment. *Nat Med*. 2014;20(7):764-8.
- Wang Q, Timberlake MA, Prall K, et al. The recent progress in animal models of depression. *Progress In Neuro-Psychopharmacology and Biological Psychiatry*. 2017;77:99-109.
- Baudry A, Mouillet-Richard S, Schneider B, et al. miR-16 targets the serotonin transporter: a new facet for adaptive responses to antidepressants. *Science*. 2010;329(5998):1537-41.
- O'Connor RM, Grenham S, Dinan TG, et al. microRNAs as novel antidepressant targets: converging effects of ketamine and electroconvulsive shock therapy in the rat hippocampus. *The International Journal of Neuropsychopharmacology*. 2013;16(8):1885-92.
- Grayson DR, Guidotti A. DNA Methylation in Animal Models of Psychosis. *Progress In Molecular Biology and Translational Science*. 2018;157:105-32.
- Seshadri M, Banerjee D, Viswanath B, et al. Cellular models to study schizophrenia: A systematic review. *Asian Journal of Psychiatry*. 2017;25:46-53.
- Viswanath B, Jose SP, Squassina A, et al. Cellular models to study bipolar disorder: A systematic review. *Journal of Affective Disorders*. 2015;184:36-50.
- Nashun B, Hill PW, Hajkova P. Reprogramming of cell fate: epigenetic memory and the erasure of memories past. *The EMBO Journal*. 2015;34(10):1296-308.
- Vierbuchen T, Ostermeier A, Pang ZP, et al. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature*. 2010;463(7284):1035-41.
- Kim K, Doi A, Wen B, et al. Epigenetic memory in induced pluripotent stem cells. *Nature*. 2010;467(7313):285-90.
- Yang N, Ng YH, Pang ZP, et al. Induced neuronal cells: how to make and define a neuron. *Cell Stem Cell*. 2011;9(6):517-25.
- Smigielski L, Jagannath V. Epigenetic mechanisms in schizophrenia and other psychotic disorders: a systematic review of empirical human findings. *Nature*. 2020;25(8):1718-48.
- Murata Y, Ikegame T, Koike S, et al. Global DNA hypomethylation and its correlation to the betaine level in peripheral blood of patients with schizophrenia. *Progress In Neuro-Psychopharmacology and Biological Psychiatry*. 2020;99:109855.
- Liu L, Wu J, Qing L, et al. DNA Methylation Analysis of the NR3C1 Gene in Patients with Schizophrenia. *Journal of Molecular Neuroscience*. 2020;70(8):1177-85.
- Santarelli DM, Carroll AP, Cairns HM, et al. Schizophrenia-associated MicroRNA-Gene Interactions in the Dorsolateral Prefrontal Cortex. *Genomics, Proteomics & Bioinformatics*. 2019;17(6):623-34.
- Turecki G, Meaney MJ. Effects of the Social Environment and Stress on Glucocorticoid Receptor Gene Methylation: A Systematic Review. *Biological Psychiatry*. 2016;79(2):87-96.
- Weaver IC, Cervoni N, Champagne FA, et al. Epigenetic programming by maternal behavior. *Nat Neurosci*. 2004;7(8):847-54.
- Weaver IC, Meaney MJ, Szyf M. Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;103(9):3480-5.
- Jensen Peña C, Monk C, Champagne FA. Epigenetic effects of prenatal stress on 11 β -hydroxysteroid dehydrogenase-2 in the placenta and fetal brain. *PLoS One*. 2012;7(6):e39791.
- O'Donnell KJ, Bugge Jensen A, Freeman L, et al. Maternal prenatal anxiety and downregulation of placental 11 β -HSD2. *Psychoneuroendocrinology*. 2012;37(6):818-26.
- Mueller BR, Bale TL. Sex-specific programming of offspring emotionality after stress early in pregnancy. *The Journal of Neuroscience: The Official Journal of the Society For Neuroscience*. 2008;28(36):9055-65.
- Hompes T, Izzi B, Gellens E, et al. Investigating the influence of maternal cortisol and emotional state during pregnancy on the DNA methylation status of the glucocorticoid receptor gene (NR3C1) promoter region in cord blood. *Journal of Psychiatric Research*. 2013;47(7):880-91.
- Oberlander TF, Weinberg J, Papsdorf M, et al. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics*. 2008;3(2):97-106.
- Radtke KM, Ruf M, Gunter HM, et al. Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor. *Translational Psychiatry*. 2011;1(7):e21.
- Blaze J, Roth TL. Exposure to caregiver maltreatment alters expression levels of epigenetic regulators in the medial prefrontal cortex. *Int J Dev Neurosci*. 2013;31(8):804-10.
- Levine A, Worrell TR, Zimnisky R, et al. Early life stress triggers sustained changes in histone deacetylase expression and histone H4 modifications that alter responsiveness to adolescent antidepressant treatment. *Neurobiology of Disease*. 2012;45(1):488-98.
- Kundakovic M, Lim S, Gudsnuik K, et al. Sex-specific and strain-dependent effects of early life adversity on behavioral and epigenetic outcomes. *Frontiers in Psychiatry*. 2013;4:78.
- Roth TL, Lubin FD, Funk AJ, et al. Lasting epigenetic influence of early-life adversity on the BDNF gene. *Biological Psychiatry*. 2009;65(9):760-9.
- Labonté B, Suderman M, Maussion G, et al. Genome-wide epigenetic regulation by early-life trauma. *Archives of General Psychiatry*. 2012;69(7):722-31.
- Labonté B, Suderman M, Maussion G, et al. Genome-wide methylation changes in the brains of suicide completers. *The American Journal of Psychiatry*. 2013;170(5):511-20.
- Cheung S, Woo J, Maes MS, et al. Suicide epigenetics, a review of recent progress. *Journal of Affective Disorders*. 2020;265:423-38.
- Melas PA, Rogdaki M, Lennartsson A, et al. Antidepressant treatment is associated with epigenetic alterations in the promoter of P11 in a genetic model of depression. *The International Journal of Neuropsychopharmacology*. 2012;15(5):669-79.
- Mora C, Zonca V, Riva MA. Blood biomarkers and treatment response in major depression. *Expert Rev Mol Diagn*. 2018;18(6):513-29.