Progress in Neuropsychopharmacology & Biological Psychiatry 81 (2018) 38–49

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

DNA methylation and antipsychotic treatment mechanisms in schizophrenia: Progress and future directions

Ellen S. Ovenden[a], Nathaniel W. McGregor[a], Robin A. Emsley[b], Louise Warnich[a,*]

[a] Department of Genetics, Stellenbosch University, Stellenbosch 7600, South Africa
[b] Department of Psychiatry, Stellenbosch University, Tygerberg 7505, South Africa

ARTICLE INFO

Keywords:
Antipsychotics
DNA methylation
Pharmacoepigenomics
Schizophrenia
Treatment response

ABSTRACT

Antipsychotic response in schizophrenia is a complex, multifactorial trait influenced by pharmacogenetic factors. With genetic studies thus far providing little biological insight or clinical utility, the field of pharmacoepigenomics has emerged to tackle the so-called "missing heritability" of drug response in disease. Research on psychiatric disorders has only recently started to assess the link between epigenetic alterations and treatment outcomes. DNA methylation, the best characterised epigenetic mechanism to date, is discussed here in the context of schizophrenia and antipsychotic treatment outcomes. The majority of published studies have assessed the influence of antipsychotics on methylation levels in specific neurotransmitter-associated candidate genes or at the genome-wide level. While these studies illustrate the epigenetic modifications associated with antipsychotics, very few have assessed clinical outcomes and the potential of differential DNA methylation profiles as predictors of antipsychotic response. Results from other psychiatric disorder studies, such as depression and bipolar disorder, provide insight into what may be achieved by schizophrenia pharmacoepigenomics. Other aspects that should be addressed in future research include methodological challenges, such as tissue specificity, and the influence of genetic variation on differential methylation patterns.

1. Introduction

1.1. Schizophrenia and treatment response

Schizophrenia is a debilitating psychiatric disorder that exhibits complex and severe symptoms (Millier et al., 2014). The disease has a global prevalence of 1%, although the types and severity of symptoms vary extensively between individuals (Curtis, 2013; Tandon et al., 2009). For the last 60 years, antipsychotics have been the only effective treatment for the disorder (Brandl et al., 2014), however, only 50% of schizophrenia patients show favourable symptom improvement (Lohoff and Ferraro, 2010; van Os and Kapur, 2009). Furthermore, even when treatment is considered successful, the negative symptoms of the disorder (including apathy, lack of motivation, and affective flattening) generally show limited improvement (Arranz and de Leon, 2007).

Antipsychotics are customarily divided into two classes, namely first generation (FGA) and second generation (SGA) antipsychotics. Both classes of drug act on the dopaminergic system as dopamine D2 receptor (DRD2) antagonists, however, SGAs are generally considered dopamine-serotonin antagonists, demonstrating additional serotonin receptor blockade (Maric et al., 2016; Miyamoto et al., 2005). In spite of this classification, some FGAs show significant serotonin antagonism, whilst certain SGAs do not, leading to the recommendation that their distinction be abandoned (Leucht et al., 2009). Furthermore, despite being preferentially prescribed, it is generally accepted that SGAs have similar efficacy to their first generation counterparts (Meltzer, 2013; Tandon et al., 2010). The difference between the two classes is most often observed when considering their associated adverse drug reactions (ADRs). FGAs, such as haloperidol and fluphenazine, can induce motor abnormalities, and SGAs, such as clozapine and olanzapine, are commonly associated with significant weight gain and other metabolic disturbances (Müller et al., 2013; Young et al., 2015; Zhang et al., 2013). These side effects are often severe and long-lasting, reducing compliance and limiting positive outcomes (Brandl et al., 2014).

A significant consideration for improving treatment outcomes is pharmacogenetics, as individual heterogeneity in symptoms, and response to treatment, is largely attributable to genetic differences (De Leon, 2009). Recently, genome-wide association studies (GWAS) have revealed a much wider spectrum of common variation associated with disorder phenotype than originally thought, including noncoding

Abbreviations: ADR, adverse drug reaction; ENCODE, Encyclopedia of DNA Elements; FES, first-episode schizophrenia; FACS, fluorescence-activated cell sorting; FGA, first generation antipsychotic; LCM, laser capture microdissection; SGA, second generation antipsychotic

* Corresponding author at: Stellenbosch University Private Bag X1, Matieland, Stellenbosch 7600, South Africa.
E-mail address: lw@sun.ac.za (L. Warnich).

http://dx.doi.org/10.1016/j.pnpbp.2017.10.004
Received 14 July 2017; Received in revised form 1 October 2017; Accepted 4 October 2017
Available online 07 October 2017
0278-5846/

variation with potential regulatory impacts (Clark et al., 2013; Liou et al., 2012; McClay et al., 2011a). Despite extensive research, there has been little functional validation or replication of GWAS results, and the biological relevance of the majority of the identified variation is unclear. Moreover, none of the variants identified thus far are sufficiently informative to predict treatment outcome (Zhang and Malhotra, 2013). Therefore, the bulk of heritability for antipsychotic response phenotypes is not explained by common genetic variation (Hindorff et al., 2009). In light of this, epigenetics has emerged as an important player in the search for the biological architecture of schizophrenia and the response to antipsychotic treatment.

With regards to both genetic and epigenetic research, published studies specifically investigating associations with treatment outcomes are much fewer than those assessing schizophrenia risk in a case-control approach. However, there is evidence for substantial overlap between loci linked to diagnosis and those implicated in treatment response, illustrated by a unique bioinformatic study conducted by Ruderfer et al., 2016. The authors compared significant loci from the latest large-scale schizophrenia GWAS to known and predicted drug targets, and observed significant enrichment for 70 known and 277 predicted antipsychotic target genes (Ruderfer et al., 2016). Furthermore, of the handful of treatment response GWAS that have been conducted, many implicate genes already associated with schizophrenia, including *DRD2* (McClay et al., 2011b), glutamate metabotropic receptor 7 (*GRM7*) (Sacchetti et al., 2017), and phosphodiesterase 4D (*PDE4D*) (Clark et al., 2013). This genetic overlap grants pharmacogenetic researchers a starting point when assessing a gene's potential relevance to treatment response and prioritising genes for further study. Therefore, even though epigenetic studies of treatment response in schizophrenia are currently limited, endeavours to uncover the epigenetic workings of schizophrenia itself, also discussed, provide additional clues about the mechanisms of antipsychotic response.

1.2. Epigenetics: focus on DNA methylation

It is clear that changes to the DNA sequence are only one aspect of schizophrenia heritability. Twin studies have demonstrated a 50% concordance rate for monozygotic twins, even though these individuals share identical genetic codes (Cardno and Gottesman, 2000; Kendler and Diehl, 1993). The complex interaction between genes and environment may, in part, account for the missing heritability observed in both the disorder itself and in response to treatment (Bell and Saffery, 2012). This interaction is mediated by epigenetic mechanisms, i.e. heritable alterations in gene regulation and expression, excluding changes to the DNA sequence itself. These mechanisms include DNA methylation, histone modifications, and regulation by microRNAs and other noncoding RNA molecules (Bird, 2007).

Epigenetics is crucial in establishing and maintaining tissue-specific gene expression patterns, and environmental changes in pre- and early post-natal periods can precipitate lifelong effects on gene regulation and expression (Abdolmaleky, 2014). Furthermore, alterations to the epigenome can be passed on to future generations, just as variants in the genetic code conveying risk for disease are inherited (Manikkam et al., 2012; Waterland et al., 2008). Epigenetic regulation is particularly important in neurodevelopment. Neuroscience research has shown its crucial involvement in brain growth, synaptic plasticity, learning, memory, and circadian rhythm (Borrelli et al., 2008; Mehler, 2008; Nakahata et al., 2007; Pidsley et al., 2010; Roth and Sweatt, 2009). For these reasons, epigenetic research within psychiatry has recently gained traction. Furthermore, there is substantial evidence hypothesising that epigenetic dysfunction is a key determinant in the development of psychiatric disorders, including schizophrenia (reviewed in Ptak and Petronis, 2010).

Studies have shown the importance of DNA methylation in the psychopathology of psychiatric disorders, as well as the potential for methylation sites as targets in drug development (Grayson and Guidotti, 2013). DNA methylation occurs predominately at CpG dinucleotides, up to 90% of which are methylated in the human genome. The remaining unmethylated sites occur in CpG-rich clusters, or CpG islands, which are present in the promoter regions of 70% of annotated genes (Jaenisch and Bird, 2003; Saxonov et al., 2006). The addition of methyl groups is catalysed by a family of maintenance and de novo DNA methyltransferases (DNMTs) (Denis et al., 2011). Only discovered in 2009, the process of active demethylation is conducted by ten-eleven translocation (TET) enzymes (Ito et al., 2011; Tahiliani et al., 2009). Traditionally, methylation is associated with repressed transcription, as the density of methylated CpGs in a promoter is frequently inversely proportional to the activity of the gene (Yeivin and Razin, 1993). This gene silencing occurs either directly via interference with transcription factor binding, or indirectly by attracting proteins that induce chromatin remodeling (Hendrich and Bird, 1998; Meehan et al., 1992).

1.3. Aberrant DNA methylation in schizophrenia

There is abundant evidence for DNA methylopathy in schizophrenia, both at the site-specific and genome-wide level. Firstly, the γ-aminobutyric acid (GABA)-ergic pathway has received much attention, and several key genes expressed in GABAergic neurons, including reelin (*RELN*) and glutamic acid decarboxylase (*GAD1*), are downregulated in schizophrenia due to promoter hypermethylation (Abdolmaleky et al., 2005; Akbarian et al., 1995; Guidotti et al., 2000; Huang and Akbarian, 2007). GABAergic dysfunction has consistently been associated with psychosis, as well as negative symptomology in schizophrenia (Bönsch et al., 2012; Hashimoto et al., 2008; Shimabukuro et al., 2006; Taylor et al., 2014), and these findings suggest that epigenetic disruptions in GABAergic genes contribute to the aetiology of the disorder. Further substantiating this, *RELN* and *GAD1* downregulation is accompanied by a concomitant increase in *DNMT1* expression in the cortex of schizophrenia patients (Veldic et al., 2005), suggesting *DNMT1* overexpression to be the driving force behind increased promoter methylation in GABAergic neurons (Grayson et al., 2009). Subsequent research has revealed upregulation of *DNMT* genes in post-mortem schizophrenia studies, including *DNMT1* (Ruzicka et al., 2007; Zhubi et al., 2009) and *DNMT3a* (Zhubi et al., 2009). Interestingly, genetic and epigenetic alterations at CpG sites of *DNMT* genes are among the most frequently found abnormalities in inherited diseases (Zingg and Jones, 1997), which emphasises the importance of DNA methylation in regulating healthy functioning.

With regards to the dopaminergic pathway, methylation studies in schizophrenia have focused on the catechol-*O*-methyltransferase (*COMT*) gene. The *COMT* promoter region demonstrated significant hypomethylation in post-mortem frontal lobe samples from schizophrenia patients compared to controls (Abdolmaleky et al., 2006). This was substantiated by increased mRNA levels and corresponded to a correlated decrease in *DRD2* expression. The authors hypothesise that upregulation of membrane-bound (MB)-COMT augments dopamine degradation and contributes to the symptoms of schizophrenia. A more recent study observed similar hypomethylation of the gene in schizophrenia and bipolar saliva samples (Nohesara et al., 2011), suggesting cross-tissue and cross-disorder relevance of *COMT* dysregulation. Given that saliva is much easier to acquire than brain tissue, this is encouraging for future research and potential biomarker development.

Another neurotransmitter system associated with schizophrenia, the serotonergic pathway, has also been investigated in the context of aberrant DNA methylation. Promoter methylation of the serotonin receptor type-1 (*HTR1A*) gene was significantly increased in the blood of bipolar and schizophrenia patients in comparison to controls (Carrard et al., 2011). Serotonin receptor downregulation has previously been linked to schizophrenia (Garbett et al., 2008; Polesskaya and Sokolov, 2002). Similarly, the type-2 (*HTR2A*) gene also demonstrated hypermethylation in the promoter region in a small post-mortem brain study (Abdolmaleky et al., 2011). Interestingly, the authors established