

Review

## Deciphering the Epigenetic Landscape of Suicidal Behaviour: A Review of Current Findings, Caveats and Future Directions

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### Abstract

Suicide is the second leading cause of death globally among young people and the tenth leading cause of death across all ages. Approximately 800,000 people die by suicide every year representing a significant global health burden. Despite this burden, the molecular pathology of suicide remains poorly understood. A number of recent studies have shown that epigenetic alterations are associated with suicidal behaviour. These epigenetic mechanisms, which act to regulate gene expression via modifications to DNA, histone proteins and chromatin, change with age and in response to specific environmental and psychosocial factors —providing a mechanism for the interaction between genotype and the environment. The present review briefly outlines the main epigenetic mechanisms involved in gene regulation and discusses recent findings of epigenetic alterations in suicidal behaviour, their caveats and the future direction of this emerging field of research.

### Keywords

Epigenetics; suicide; DNA methylation; histone modification; non-coding RNA



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## 1. Introduction

Suicide is a serious public health problem that can have lasting harmful effects on individuals, families, and communities [1]. Suicide is the second leading cause of death among 15–29 year olds worldwide and ranks amongst the 20th leading causes of death across all ages [2]. Approximately 800,000 people die by suicide every year [2] representing a significant global health burden. Suicidal behaviour (SB) spans a spectrum ranging from suicidal ideation (SI) to suicide attempt (SA) and completed suicide [3, 4]. Up to one-third of individuals with SI have a SA within 1 year; individuals who have had a SA have 1.6% risk of suicide within the year [5]. Despite its economic and social burden, the underlying aetiology of SB remains poorly understood. Genetic epidemiological studies have found evidence of family clustering of SB and have highlighted a clear genetic susceptibility to SB [6, 7] with heritability estimates ranging from 30-50% [8-10]. A significant proportion of the heritability for SB and SA appears to be independent of psychiatric diseases as when the heritability of SA is corrected for transmission of psychiatric disorders, specific heritability is between 17% and 36% [8, 11-15].

Pre-existing knowledge of biological systems likely to be associated with SB, such as serotonergic neurotransmission and neurotrophic factors, resulted in several candidate-gene association studies [7, 14-17] which have largely yielded inconsistent results. Similarly, large-scale genome-wide association studies (GWAS) [18-20] have failed to identify robust associations suggesting that the risk of SB is highly polygenic in nature and that individual gene variants are likely to account only for a very small proportion of the total phenotypic variability. Other factors, such as the environment, behavioural traits, lifestyle and coping mechanisms, are essential regulators of suicide risk and likely to account for more sizeable effects [21]. Recently, increased understanding of epigenetic processes that occur in the brain has opened promising avenues in suicide research. The epigenome is potentially malleable—changing with age [22] and in response to specific environmental [23] and psychosocial factors [24] —providing a mechanism for the interaction between genotype and the environment [25]. Epigenetic processes, including DNA methylation, have recently been implicated in the aetiology of numerous mental health disorders [26-33] and SB [34, 35]. The present review will briefly introduce epigenetic mechanisms, focussing primarily on DNA methylation, histone modifications and non-coding RNAs and explore their relevance to suicide and SB, before discussing future directions and caveats of this emerging field of research and its potential to further our understanding of SB.

### **1.1 Beyond Genome-Wide Association Studies: a Role for Epigenetic Variation in Suicide and SB**

The term epigenetics, literally meaning ‘above’ genetics, was originally coined to refer to the study of any potentially stable and heritable changes in gene expression or cellular phenotype that occurs without changes in Watson–Crick base-pairing of DNA [36]. However, the epigenome, which is the chemical structure surrounding our genome, can alter the function of our genes in response to the environment [37], and remains dynamic throughout life [37-39].

Epigenetic variation is regulated in a tissue-specific manner by stochastic, genetic and environmental factors [40]. In the brain, epigenetic variation is thought to influence the gene

expression profile of neurons and glial cells [41, 42]. Therefore it is possible that alterations of epigenetic profiles in the brain may induce long-term behavioural consequences. As such they have the potential to play a crucial role in determining predisposition to SB [43]. The most commonly studied epigenetic mechanisms that can influence gene expression are: molecular modifications of the DNA sequence, post-translational histone modifications and non-coding RNA gene silencing [44].

### **1.2 DNA Modifications**

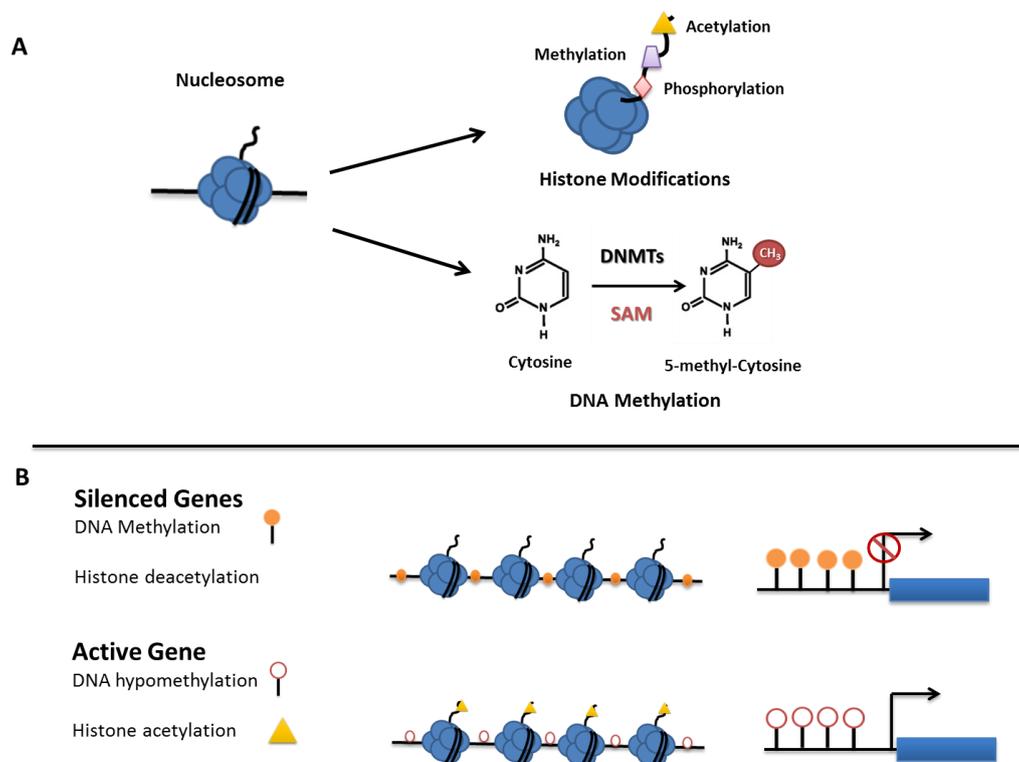
DNA methylation is the covalent modification at the C5 carbon of cytosine residues, occurring mainly at CG dinucleotides (CpG) (See Figure 1). In somatic cells, approximately 80% of CpGs are methylated [45]; the remaining unmethylated CpGs tend to be concentrated around gene promoters (CpG islands) [37]. A Recent study has reported 5-methyl cytosine (5mC) to be central to the establishment of tissue-specific gene expression, cell differentiation, genomic imprinting, and X-inactivation [46]. When found in promoter regions of genes, CpG methylation is generally associated with transcriptional repression or downregulation of RNA transcription [47]. However, DNA methylation has also been described in other regions of the genome and the transcriptional effect in those areas is not consistent [24]. Previous research has suggested a role for global DNA methylation as a potential marker of suicide risk. A significantly higher level of global methylation (5mC %) has been reported in peripheral blood of psychiatric patients with a history of SA when compared with psychiatric controls [35]. Similarly, an increase in DNA methylation in the brain region BA47 was found to be 8 times greater in the group who died by suicide relative to controls [44]. Taken together these findings, although requiring further replication, suggest that global DNA methylation may be a biomarker of suicide risk in psychiatric patients and support the idea that blood tissue may be reasonably considered a valid proxy to monitor brain changes.

Although DNA methylation has been considered as a stable epigenetic mark, studies in the past decade have revealed that this modification is not as static as once thought. Recently, research has provided evidence that 5mC can be oxidized to 5-hydroxymethylcytosine (5hmC) in a reaction catalysed by the ten–eleven translocation (TET) enzymes [48-50] along with two other oxidative products: 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). Growing evidence suggests that these demethylation states may play an important biological role [51, 52].

### **1.3 Histone Modifications**

Chromatin exists as two functional structures: euchromatin which is the “opened” state associated with gene transcription and heterochromatin which is the “closed” state associated with gene silencing. Proteins forming this complex structure are called histones (H2A, H2B, H3, and H4). These globular proteins, with a tail of amino-acids, can be covalently modified by the addition or the removal of chemical groups at specific residues on their N-terminus tails (See Figure 1) [37]. Histone modifications are able to alter the access of transcriptional machinery to the DNA by modifying the condensation of chromatin [37]. Although there are several molecular modifications histone acetylation/deacetylation and methylation are the two most commonly studied.

Studies examining the role of histone modifications in SB are limited to the study of candidate genes [53, 54], thus genome-wide approaches have not yet been undertaken.



**Figure 1** Epigenetic modifications and transcriptional regulation. (A) Figure illustrates common modifications that occur on the tails of histone proteins and the conversion of Cytosine to 5-methyl-Cytosine by a group of enzymes called DNA methyltransferases (DNMTs) and S-Adenosyl-L-methionine (SAM). (B) Transcriptionally inactive genes are generally characterised by DNA Methylation at CpG dinucleotides in the promoter region of genes and histone deacetylation. In contrast, transcriptionally active genes are generally characterised by absence of DNA methylation (DNA hypomethylation) and histone acetylation.

### 1.4 Non-Coding RNAs

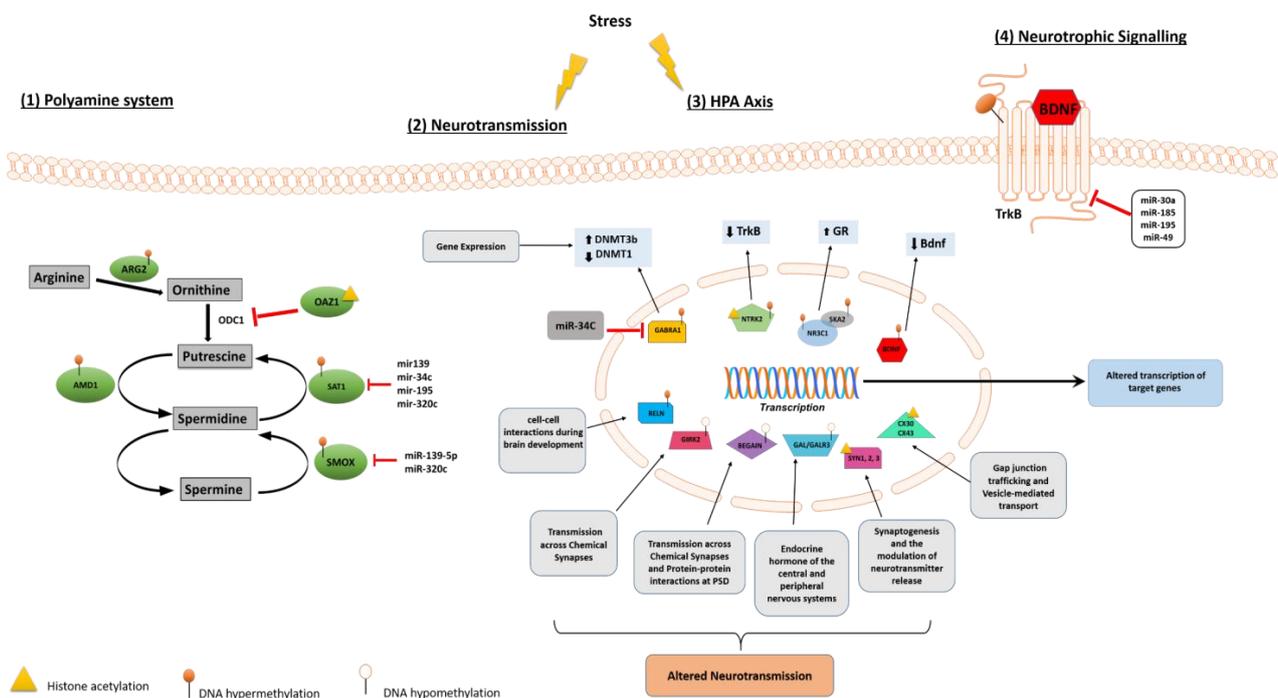
Another mechanism of epigenetic regulation of gene expression is mediated by non-coding RNAs (ncRNAs), functional RNA molecules that are transcribed from DNA but not translated into proteins. These regulatory RNAs function to regulate gene expression at the transcriptional and post-transcriptional level. Several classes of ncRNAs exist, including microRNAs (miRNAs), short interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs) also referred to as short ncRNAs (<30 nts) to distinguish them from the long non-coding RNAs (lncRNAs) (>200 nts). Both major groups are shown to play a role in heterochromatin formation, histone modification, DNA methylation targeting and gene silencing [55]. To date, miRNAs are the best characterised ncRNA group. They act as regulators of gene expression and protein translation in many tissues, including brain, by binding to the target mRNA and contributing to its degradation through the RNA-induced silencing complex (RISC) complex or by directly inhibiting translation [56]. MiRNAs are known to play a critical role in developmental processes, including neurodevelopment and synaptic plasticity and they have been implicated in the pathogenesis of various neuropsychiatric disorders like schizophrenia, major depression and bipolar disorders [55-58]. The specific dysregulation of miRNA function in suicide is just beginning to be appreciated, as recently reviewed [56].

Cataloguing the expression of miRNAs and additional small RNAs in healthy human brain as well as in major neuropsychiatric diseases and suicide may lead to new therapeutic targets and insight into disease aetiology [55].

LncRNAs represent another interesting class as they are enriched for expression in the brain [59] and are developmentally regulated [60] but less evolutionarily conserved than other RNA species. Although preclinical studies have begun to unravel how lncRNAs may contribute to emotional control [61], their role in SB is currently unknown.

## 2. Common Neurobiological Pathways Epigenetically Dysregulated in Suicide

In the last decade, understanding the role of epigenetic mechanisms in SB has implicated the role of key biological pathways, including hypothalamic pituitary adrenal (HPA) axis, stress response, polyamine system, neurotrophic signalling and lipid metabolism. A comprehensive overview of genes epigenetically dysregulated in suicide and SB is reported in Table 1 and Table 2. Common pathways frequently targeted by epigenetic and miRNA dysregulation are illustrated in Figure 2 and discussed in detail below.



**Figure 2** Molecular pathways involved in suicide behaviour. (1) Polyamine system: several enzymes involved in the regulation of polyamine intracellular concentration exhibit epigenetic regulatory mechanisms in suicide through DNA methylation (*AMD1*, *ARG2*), histone methylation (*OAZ1*) and/or post-translational mechanisms (*SAT1*, *SMOX*) as miRNAs regulation. (2) Neurotransmission: many genes known to play a key role at chemical and/or electrical synapses have been reported as hypomethylated (*BEGAIN*, *GIRK2*, *GALR3*), hypermethylated (*RELN*) or histone methylated (*SYN1-3*, *CX30*, *CX43*) in suicide cases compared to healthy controls suggesting that neurotransmission may be compromised in suicide. (3) HPA axis: two genes differentially involved in cortisol release following stress (*NR3C1*, *SKA2*) have been

found to be both significantly hypermethylated in suicide cases relative to controls supporting the hypothesis of suicide-associated HPA axis dysregulation. (4) Neurotrophic Signalling: several studies examining *BDNF* and *TrkB* in post-mortem suicide brains revealed distinct epigenetic regulation pattern for these two genes: the receptor *TrkB* seems to be regulated via miRNAs (miR-30a, miR-185, miR-195 and miR-49) and histone acetylation while the expression levels of the neurotrophin *Bdnf* correlate with DNA hypermethylation in the promoter region of the gene.

## **2.1 HPA Stress Axis**

It is well established that perturbations in the HPA axis are associated with the pathophysiology of suicide [62]. The HPA axis is a neuroendocrine system, key regulator of cortisol release and the stress response [63]. Research has shown that the HPA axis may be epigenetically programmed by the early-life environment [64]. Several HPA-axis coupled genes have been identified over the years: corticotropin releasing hormone (*CRH*), corticotropin releasing hormone binding protein (*CRHBP*), corticotropin releasing hormone receptor 1 (*CRHR1*), corticotropin releasing hormone receptor 2 (*CRHR2*), FK506-binding protein 51 (*FKBP5*) and the glucocorticoid receptor (*NR3C1*)[65].

More recently, a study aiming to identify DNA methylation shifts linked to severity of the SA in HPA-axis coupled genes was conducted in whole blood derived DNA. The authors reported reduced levels of DNA methylation at two loci within the *CRH* promoter in serious suicide attempters. Interestingly, significant blood-brain correlations in DNA methylation were also found suggesting that these alterations may impact on expression profile of *CRH* in the brain [65].

Glucocorticoids. Glucocorticoids play a crucial role in the maintenance and survival of neurons and in synaptic plasticity. A study examining the DNA methylation status of *GR* exon 1F variant in individuals who died by suicide and were severely abused during childhood observed increased DNA methylation at the *GR* promoter compared to suicide victims with no childhood abuse or controls [66]. Moreover, *GR* methylation status appears to regulate the binding of the *NGFI-A* transcription factor associated with the *GR* expression [66, 67]. The disrupted *GR* function may result in inadequate control of the HPA axis, possibly leading to hyperactive cortisol secretion and development of anxiety traits. In turn, anxiety mediates the relationship between the exposure to early life adversity (ELA) and SB [64],[68]. More recently, a study has suggested a role for dietary deficiencies in mediating the interaction between an altered *GR* system and SB. DNA methyltransferases catalyse the transfer of a methyl group from the methyl donor, *s*-adenosylmethionine (SAM), onto the 5C position of the dinucleotide sequence CG. However, the synthesis of SAM is dependent on the availability of dietary foliates, vitamin B12 and choline, suggesting an alternative mechanism to altering the expression of the *GRII* exon 17 promoter [69].

SKA2 - Altered microtubule binding. An exciting new candidate in the relationship between cortisol regulation and suicide is the spindle and kinetochore associated protein 2 (*SKA2*), a gene that has been implicated in *GR* signalling [70]. This microtubule binding protein is thought to interact with the HPA axis by chaperoning the *GR* from the cytoplasm to the nucleus upon cortisol binding [70]. Once in the nucleus, the *GR* can interact with genomic DNA and influence gene expression involved in negative feedback regulation of the HPA axis response. In 2014, a study identified site and allele-specific DNA methylation patterns in the *SKA2* gene, in particular

increased *SKA2* 3' untranslated region methylation and concomitantly decreased *SKA2* mRNA levels, were detected in the frontal cortex of suicide completers [71]. Further research found that *SKA2* DNA methylation predicted lifetime SA in saliva and blood in individuals suffering from post-traumatic stress disorder (PTSD) [72]. Higher DNA methylation at the same site and allele was also found to predict lower levels of miR-301a in the cortex of depressed suicide completers [73]. Since the expression of this miRNA is tied to *SKA2* expression, it was suggested as a proxy of suicide-associated *SKA2* decreases [55, 72]. Further support to these preliminary findings has been provided by more recent studies suggesting the influence of trauma exposure on *SKA2* DNA methylation as well as DNA methylation of the *SKA2* gene as a biomarker of suicide risk and stress-related psychopathology [74, 75].

## **2.2 Polyamine System and *SAT1***

The polyamine system is another stress response pathway which has been extensively characterized in relation to suicide risk. Polyamines, aliphatic compounds with multiple amine groups, have been implicated in regulation of gene expression at transcriptional and posttranscriptional levels, regulating the function of several neuromodulators and acting as neurotransmitters themselves [76, 77]. Involvement of epigenetic modifications in the regulation of genes associated with polyamine biosynthesis has been implicated in SB [78].

*SAT1* is an enzyme involved in polyamine catabolism and is arguably one of the most consistently downregulated gene in depressed suicide completers [79-85]. A potential mechanism for *SAT1* downregulation is through epigenetic control, as studies have identified that *SAT1* promoter DNA methylation is inversely correlated with *SAT1* expression. Moreover, there is evidence for histone modifications affecting *SAT1* expression along with other key enzymes in polyamine synthesis [54, 78, 82] and that miRNAs can target polyamine transcripts, including *SAT1* [86]. *SAT1* has therefore emerged as a potential biomarker for suicide, topping the lists of candidate genes in several studies [84, 87, 88]. However, it is still unknown whether this gene is involved in major depressive disorder (MDD) independently of suicide, which isoforms are dysregulated and whether the gene undergoes differential splicing in suicide and depression [89].

The expression of several other polyamine-associated enzymes were found altered in the cortex of post-mortem suicide cases. The spermine oxidase (*SMOX*) gene, encoding for a catabolic enzyme, was found hypermethylated in the promoter region in the prefrontal cortex (PFC) of suicide subjects. However, there was no correlation between aberrant promoter DNA hypermethylation and gene expression changes [83]. Two studies have identified DNA hypomethylation at the promoter region of the arginase 2 (*ARG2*) and adenosylmethionine decarboxylase1 (*AMD1*) genes, which correlated with increased gene expression in suicide completers compared to controls [54, 78]. A different epigenetic modification showed to regulate the Ornithine Decarboxylase Antizyme 1 (*OAZ1*) gene, also involved in the intracellular regulation of the polyamine levels. Increased H3K4me3 levels in the promoter region of *OAZ1* were found in suicide completers and appeared to correlate with the expression of *OAZ1* and *ARG2* in Brodmann area 44 [54]. Taken together this research provides support for the involvement of epigenetic modifications in the regulation of genes associated with polyamine biosynthesis, which may play an important role in SB.

### **2.3 Neurotrophic Pathways - BDNF and NTRK2**

Genome-wide DNA methylation studies in the brains of suicide completers indicates that suicide is associated with widespread changes in DNA methylation patterns of neurotrophic and neuroprotective factors in the hippocampus and PFC [40, 90]. Brain derived neurotrophic factor (BDNF) is a well-known receptor binding factor with growth factor activity; it is essential for the survival and plasticity of cortical and striatal neurons and it has been implicated in both stress response [17] and mood disorders [91]. Increased *BDNF* promoter/exon 4 DNA methylation has been reported in suicide brains [92], a finding that is consistent with those observed in depressed patients with a history of SA, or with SI during treatment with antidepressants [93]. Similarly, another study focusing on the Wernicke area of suicide subjects, reported a remarkable increase of DNA methylation at the *BDNF* promoter IV in suicide subjects with respect to controls [43]. Taken together this evidence suggest that *BDNF* DNA methylation status maybe a proxy marker for previous suicidal attempts and a clinical biomarker for poor treatment outcomes of SI in depression. However, larger studies are required to confirm these findings.

Interestingly, TrkB, main receptor of BDNF and encoded by the *NTRK2* gene, is also regulated through epigenetic changes that appear to have an impact on suicide risk. In brain tissue from individuals who died by suicide, mRNA expression of *TrkB-T1*, the astrocyte-enriched TrkB truncated variant, was found significantly decreased and correlated with increased DNA methylation at the *TrkB-T1* promoter suggesting that astrocytic dysfunction may occur in individuals who die by suicide [53, 94, 95].

### **3. Non-Coding RNAs and Their Role in SB**

Recently, a number of studies have implicated a role for miRNA dysregulation in the pathogenesis of SB (see Table 2 for full list of suicide-associated miRNAs). Interestingly, some of the suicide-associated genes previously discussed as showing altered DNA methylation and/or histone modifications were found to be targets of differentially expressed miRNAs. For example, the expression levels of two genes involved in the polyamine metabolism (*SAT1* and *SMOX*) were found to be downregulated by overexpression of miRNAs (e.g. miR-139, miR-34c, miR-195, miR-320c) [86, 89] in suicide cases compared to controls. Similarly, miR-185, which is thought to regulate the *NTRK2* gene (TrkB receptor) is differentially expressed in the frontal cortex of suicide completers [96]. Furthermore, miR-185, together with miR-195 and miR-30a have been implicated in regulating the BDNF signalling pathway [56, 96, 97], a pathway widely reported as involved in SB. Taken together, these findings suggest a co-ordinated epigenetic and miRNA regulation of pathways relevant to SB.

Sun *et al*, implicated a role for notch signalling in SI in a study of miRNAs from peripheral blood leukocytes of Chinese MDD patients [98]. The expression levels of two notch-associated miRNAs, miR-34b–5p and miR-369–3p, were found to be significantly lower in patients with SI relative to patients without SI [98]. Consistent with these results, expression levels of these miRNAs have been previously shown to be reduced in PFC of depressive suicidal patients compared to normal controls [73], highlighting the potential utility in examining changes in miRNA expression in blood in individuals with SB.

In addition to miRNAs, expression levels of lncRNAs have also been implicated in suicide. Recently, our group implicated a role for differential DNA methylation at the *PSORS1C3* locus, a

non-coding RNA, in the brains of suicide completers. Although the function of the *PSORS1C3* gene product remains unknown, it is thought to potentially regulate nearby genes (for example, *POU5F1* and *HLA-C*), suggesting a role in immune system regulation. Moreover, *PSORS1C3* is a known psoriasis susceptibility gene further supporting a role in immune system regulation [34]. Furthermore, a recent study identified six lncRNAs (see Table 2) whose expression was downregulated in peripheral blood samples of MDD patients with SI [99]. Finally, Punzi *et al* showed that the expression of an uncharacterized lncRNA, *LOC285758*, is significantly increased in violent suicide cases compared to non-violent suicide cases [100]. Interestingly, DNA methylation of an intragenic CpG island in the myristoylated alanine-rich protein kinase C substrate (*MARCKS*) gene, a gene previously reported [84] as potential blood biomarker for suicidality, was found to be associated with the expression of this lncRNA, suggesting that the previously reported *MARCKS* association with violent suicide involves regulation by *LOC285758* expression. In conclusion, profiling ncRNAs can substantially contribute to our understanding of how gene expression networks are reorganized in suicide.

#### **4. Current Methodological Caveats in Suicide Epigenetic Research**

##### **4.1 Phenotype Assessment**

The wide range of phenotypes that may be considered in studies investigating SB further complicate identification of clear biological markers by hampering the comparability of studies, even among studies adopting similar approaches. SI and SA may at times be studied concurrently. These phenotypes are often considered to exist on a spectrum and, as a result, are frequently studied and reported on together in genetic and epigenetic studies. A genetic component has been demonstrated for SA and completed suicide but the role of genetics in SI is currently unclear [11]. Thus, it has been suggested to exclude SI from future SB epigenetic studies [3]. SA ranges from highly lethal, to low lethality for failed SA, and could be divided according to impulsive characteristics or chronic SA. However these two phenotypes, completed suicide and SA, are likely to only partly share underlying etiological and neurobiological mechanisms [101] suggesting therefore the need for a revised and more accurate definition of SA phenotype, which includes lethality, in order to ensure inter-study comparability. Finally, SB is generally a complication of a psychiatric disorders like MDD, schizophrenia and/or bipolar disorder. How to distinguish suicide diathesis-related epigenetic changes from those associated with mood disorders and other suicide-associated psychiatric diseases is a crucial issue. Future epigenetic studies need to examine potential SB-related epigenetic modification in a suicidal population with and without psychiatric disorder comorbidity.

##### **4.2 Tissue Heterogeneity**

Compared with genetic studies, epigenetics studies are constrained by several additional biological and methodological design issues. A major challenge that distinguishes epigenetic and genetic studies is the choice of tissue type [25]. In contrast to genetic studies, epigenetic marks are by definition tissue and cell type specific, thus tissue choice for epigenetic studies of complex traits, requires careful consideration [25]. As almost all tissues consist of multiple epigenetically distinct cell types, a major concern in epigenetic epidemiology studies is that any apparent

disease-associated epigenetic differences may simply reflect differences in cellular composition [25]. Furthermore, failure to account for this cellular heterogeneity could limit the power of epigenetic studies in complex phenotypes. For whole blood, routine cell counts [102] or the use of algorithms that can infer cellular composition from epigenomic data [103] can be applied to control for this variation statistically, and similar approaches have been developed for other heterogeneous tissues, such as the brain [104]. Approaches such as laser capture microdissection (LCM) or using fluorescence-activated cell sorting (FACS) have been recruited to detect disease-associated changes that are manifest in only a small subset of cells, which may not be detectable in analyses of whole tissue. However, manual sorting [105] and LCM [106] are useful for isolating small numbers of cells but do not provide enough material for epigenomic studies. FACS can isolate larger numbers of cells but may be challenging in tissues such as the adult brain, where cells are morphologically complex and densely interconnected. To overcome these challenges nuclei can be isolated from post-mortem tissue and then sorted using FACS for cell type specific epigenomic /transcriptomic studies [107-110]. However, FACS-sorted nuclei are fragile and difficult to concentrate into the small volumes that are optimal for chromatin assays or to keep intact for DNA methylation assays. Another major challenge is selecting nuclear expressed cell-type specific markers for sorting. Although several cell-specific markers have been identified and validated over the years, most of them are expressed in the cytoplasm or on the cellular membrane making them unsuitable for nuclei sorting. Neuronal nuclear antigen (NeuN) is commonly used as a robust marker of neurons and its application in flow cytometry analyses have been widely validated by several recent studies in mouse and human post-mortem brain samples [104, 111-114]. Although NeuN is specific to neurons, not all neurons express NeuN (e.g. cerebellar Purkinje neurons, olfactory bulb mitral cells, cortical Cajal-Retzius neurons, neurons of the inferior olive and dentate nuclei) [115, 116]. Moreover, no robust nuclear markers suitable for FACS are available to date for mature oligodendrocytes, astrocytes and microglia isolation from human brain tissue.

Further research aiming to identify new markers for nuclei labelling is needed to separate nuclei from different brain regions as well as different cell-types and neuronal sub-types unique to different cortical layers. This would empower research discoveries on nuclear changes occurring during disease progression, especially in rare but important populations, such as immune cell types present in the brain, which are thought to be critical in neuroprotective and neuroinflammatory processes [117].

#### **4.3 True Methylation Assessment**

5hmC plays a key role in the brain, where it is particularly abundant and dynamic during development [118, 119]. Current molecular approaches used to measure levels of DNA methylation (including sodium bisulphite conversion methods) do not distinguish between 5mC and 5hmC [120] suggesting that densely hydroxymethylated regions of DNA may be underrepresented in quantitative methylation analyses. However, several methodologies have now been developed to address this issue (including oxBS-Seq [121], mTAB-Seq [122] or Aba-Seq [123]). 5mC and 5hmC seem to have opposite relationships with transcriptional activity, with 5mC negatively correlating with gene expression [124] and 5hmC positively correlating with gene expression in the rodent [125] and human brains [118]. Although many studies showed that 5hmC

is linked with neurological disorders such as Alzheimer's and Huntington's diseases [126, 127] and psychiatric disorders disorder [128-130] its potential implication in SB remains unknown.

#### **4.4 Caveats of miRNA Profiling**

Expression profiling of miRNAs have increased our understanding of which miRNAs are present in specific tissues and how they may change under pathological conditions [131]. However, once identified, linking a miRNA to its mRNA targets can be a challenging task, and the mRNA target pairs should be validated. As reviewed elsewhere [132], a very small fraction of software-predicted miRNA targets are validated *in vivo* and many databases for miRNA target prediction are being developed in order to address this issue [132]. However, those databases can provide a surprising level of divergent results when we take into account degrees of sequence similarity, conservation, site accessibility, and variation in the targeted regions of the mRNA. Similarly, there are a number of conflicting studies with regard to the magnitude and direction of biologically-relevant miRNA expression changes in psychiatric disorders [133, 134]. This could be due to tissue-specific variation in expression levels as well as heterogeneity in quantification and normalization procedures [135]. Furthermore, some studies on miRNAs and depression were conducted in peripheral blood despite uncertainties regarding how closely changes in peripheral miRNA expression reflect modifications in the central nervous system (e.g., [136]). Finally, it should be also considered that "control" RNAs commonly used to normalize miRNA data (U6, U44, and U48) are very sensitive to post-mortem decay [132].

#### **4.5 Sample Size and Peripheral Tissue Profiling**

The abundance and availability of brain samples is a big challenge and many studies are relying on a limited number of brain samples reducing the power of epigenome-wide association studies [40]. Only genes that have been replicated in independent methylation studies should be considered further as primary candidate genes. Although it is desirable to increase sample sizes in the next few years, it is unfeasible to reach the numbers of samples currently being analysed in GWASs using human brain [40]. The small sample size of most of the current studies also represents a limitation when it comes to identifying gender-specific epigenetic changes associated to SB. Studies are looking towards the use of peripheral tissues (e.g. whole blood, saliva, cerebrospinal fluid (CSF)) to model a brain-related phenotype. Given the tissue-specific nature of the epigenome, the assessment of disease-relevant tissue is an important consideration for EWASs. Although some studies suggest that peripheral samples (e.g. CSF) would adequately model brain gene expression changes, little is known about whether easily accessible tissues, such as whole blood, can be used to address questions about inter-individual epigenomic variation in inaccessible tissues, such as the brain [137]. Epigenetic marks are more variable between different tissues of the same individual than between the same tissue of different individuals. There is however evidence of within-individual epigenetic variation correlation across tissues [138]. A recent study explored co-variation between tissues and the extent to which methylomic variation in blood is predictive of inter-individual variation identified in the brain. Their data suggest that for the majority of the genome, a blood-based EWAS for disorders where brain is presumed to be the primary tissue of interest will give limited information relating to underlying pathological

processes [137]. However, the authors do not discount the utility of using a blood-based EWASs to identify biomarkers of disease phenotypes manifest in the brain [137].

#### **4.6 Animal Models**

To date no convincing animal models of suicide have been produced [4]. Suicidal behaviour is challenging to detail in a non-human model as the underlying pathophysiology is poorly known and the risk for suicidal acts is multi-factorial, consisting of a range of biological (e.g. genetics), psychiatric, psychosocial, interpersonal and cultural risk factors. The most promising endophenotypes worth investigating in animals are the cortisol social-stress response and the aggression/impulsivity trait, involving the serotonergic system as well as neurotrophic factors and neurotrophins which have been implicated in suicide previously [4]. Future animal studies could focus on elucidating the effect of suicide-associated epigenetic changes on gene function and how this might impact known suicide-associated endophenotypes and biological pathways.

#### **4.7 Causality**

Currently it remains unclear whether the epigenetic changes observed cause SB and suicidal acts or if these phenotypes (or underlying psychiatric conditions/medication) cause the detected epigenetic changes. In the attempt to answer this question, Mendelian randomization (MR) is proving to be a promising approach. MR posits that if a biomarker is causally related to a disease or phenotype, genetic variant(s) controlling activity of that biomarker should also be associated with the outcome [139, 140]. Using genetic variants as proxy for exposure overcomes confounding because genetic variants are inherited at random during meiosis, so they are unrelated to potential confounders (measured or unmeasured). Therefore, following the principles of MR may represent a valid method for revealing the role of specific genes as modifiers/risk factors of SB, leading to a possible association between suicidality and a given biomarker. Future studies examining epigenetic alterations in SB should be undertaken by applying these novel approaches in order to investigate the direction of effect. For instance, the 2-step epigenetic MR approach, which is an extension of MR assumptions, allows researchers to investigate the causal role of DNA methylation in the association between an environmental exposure and SB [141, 142].

None of the studies outlined in this review have attempted to examine the causal pathways between epigenetics changes and SB, either statistically or experimentally. Recent advances in CRISPR-Cas9 based systems enable researchers to direct epigenetic machinery to specific locations with possibly greater ease and at a lower cost. Once the major drawbacks with dCas9 have been solved (such as the inefficiency of precise base editing and off-target activities), *in vivo* epigenome editing methods will potentially allow for more labs to investigate the behavioural outcomes of specific epigenetic patterns in cell or animal models. Although most of the excitement around CRISPR to date has been focused on the ability of genome editing to cure disease, epigenome editing methods might enable more subtle and reversible modulatory control [24]. Future studies will greatly benefit from these new tools and allow for a precise investigation of the direct molecular and behavioural outcomes of suicide-induced epigenetic patterns in the brain.

#### **4.8 Other Considerations**

Typically, post-mortem studies report post-mortem interval (PMI), which represents the amount of time between a subject's death and collection and processing of the brain. It has previously been shown that DNA, miRNAs and some histone modifications are stable across extended PMIs [143-146]. Moreover, a recent study has reported that neither global nor site-specific levels of 5mC and 5hmC are affected by the post-mortem intervals [46].

A further complication stems from the emergence of SI during antidepressant treatment. There is indeed a longstanding belief that antidepressants might have an early "triggering effect" that induce depressed patients to pursue suicidal impulses before the treatment-induced mood improvement [147]. Some patients being treated for depression and other psychiatric illnesses experience suicidal thoughts and actions (suicidality) as previously reported in a small proportion of patients receiving selective serotonin reuptake inhibitors [147]. Therefore, when medication data are not available for all individuals included in a study, the possibility that the observed epigenetic changes are confounded by medication cannot be ruled out.

#### **5. Future Perspectives**

A multi-omics approach to disentangle the molecular basis underlying SB is warranted to further our understanding of suicidality and SB. Genome-wide investigations have demonstrated that the genetic predisposition to complex traits is highly polygenic. The common variants detected by polygenic risk score analyses may help to predict the extent to which the genetic predisposition for suicidal acts has influence on the risk of suicide attempts. By integrating polygenic risk scores for SA, DNA modifications, chromatin modifications and miRNA expression profiling together would allow the research community to gain a broader and deeper understanding of the pathways primarily involved in the neurobiology of suicide and their potential co-ordinated interaction. For the same reason, future studies should also examine the transcriptional consequences of the observed suicide-related DNA methylation changes. System biology methods, such as weighted correlation network analysis (WGCNA), could be applied to identify suicide-associated gene pathways and networks that could be further targeted as biomarkers or therapeutic targets for suicide prevention.

#### **Author Contributions**

All authors conceived the topic of the review. SP prepared the first draft of the review and ELD and TMM edited subsequent versions of the draft. SP and TMM prepared and conceived Figure 1 and Figure 2.

#### **Competing Interests**

The authors have declared that no competing interests exist.

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**Table 1** Aberrant epigenetic changes associated with suicidal behavior

GENE SYMBOL	PROTEIN ENCODED	GENE FUNCTION (NCBI)	MOLECULAR FUNCTION (UniProt)	TISSUE	KEY FINDINGS	REFERENCES (PMID)
<b>DNA methylation</b>						
<i>Global DNA methylation</i>	NA	NA	NA	BA47	8-fold increased DNA methylation in suicide group compared to controls	25364291*
<i>Global DNA methylation</i>	NA	NA	NA	Peripheral blood	Psychiatric patients with a history of SA had significantly higher levels of global DNA methylation compared with controls	23025623* <sup>§</sup>
<i>NR3C1</i>	Glucocorticoid receptor	Maintenance and survival of neurons and in synaptic plasticity	Affects inflammatory responses, cellular proliferation and differentiation in target tissues. Involved in chromatin remodelling. Plays a role in	Hippocampus	Significant DNA hypermethylation at <i>GR</i> promoter in the hippocampus of suicide completers with history of abuse compared to controls	19234457* <sup>§</sup>
				Hippocampus	DNA hypermethylation in the exon 1F of the <i>GR</i> gene in abused individuals who died by suicide compared to non-abused individuals	22752237*
<i>RNR1</i>	ribosomal RNA	Critical functions in the ribosome that allow protein synthesis to occur	NA	Hippocampus	Overall DNA hypermethylation of rRNA promoter in the hippocampus of suicide completers with history of abuse/childhood adversity	18461137*

<i>NTRK2</i>	TrkB-T1 (astrocytic variant)	Receptors of neurotrophins. Role in neurotransmission, calcium release, synaptic plasticity, and cell survival. High affinity receptor of BDNF associated with mood disorders and SB	Development and the maturation of the central and the peripheral nervous system	PFC (BA 8/9)	Increased DNA methylation in two CpG sites within the promoter of TrkB-T1 accompanied by decreased mRNA expression in the PFC of suicide completers compared to controls	19124685
				PFC (BA 8 / 9)	DNA hypermethylation at four CpG sites in the TrkB-T1 3' UTR region in suicide cases compared to controls. Correlation between DNA methylation levels at these sites and TrkB-T1 expression	24802768*
<i>GABRA1</i>	GABAA $\alpha$ 1	Stabilizing or hyperpolarising the resting potential Inhibitory effect, reducing the activity of the neurons	Drug binding, GABA-A receptor activity, GABA-gated chloride ion channel activity	FPC	Increased DNA methylation detected in the <i>GABRA1</i> gene and negatively correlated with <i>DNMT3B</i> protein expression but positively with <i>DNMT1</i> mRNA in the FPC of suicide cases	18639864
<i>RELN</i>	reelin, glutamic acid decarboxylase	Cell-cell interactions control, critical role in cell positioning and neuronal migration during brain development	Lipoprotein particle receptor binding, metal ion binding, protein kinase activity	Occipital cortex	DNA hypermethylation at the <i>RELN</i> promoter in SZ subjects who died by suicide compared to controls	15961543
				Forebrain	Hypermethylation at 3 CpG sites within <i>RELN</i> promoter in SZ suicide cases compared to controls. DNA hypermethylation negatively correlated with reelin expression	17310238

<i>SAT1</i>	Diamine acetyltransferase 1	Enzyme in polyamine catabolism	Polyamine metabolism	PFC (BA 8/9)	DNA hypermethylation in suicide completers carrying the C allele at the rs6526342 SNP within the <i>SAT1</i> promoter region. CpG methylation at <i>SAT1</i> promoter negatively correlates with gene expression	21501848*
<i>SMOX</i>	spermine oxidase	Enzyme in polyamine catabolism	Role in the regulation of polyamine intracellular concentration	PFC	DNA hypermethylation in the promoter of <i>SMOX</i> in the brain of suicide subjects. No correlation with the gene expression levels	20059804*
<i>BDNF</i>	brain derived neurotrophic factor	Role in neuronal survival and plasticity, in the regulation of the stress response and in the biology of mood disorders	Growth factor activity, neurotrophin, TRKB receptor binding	Wernicke area	<i>BDNF</i> promoter IV hypermethylation in the suicide brains compared to controls. DNA hypermethylation levels correlates with significantly lower <i>BDNF</i> expression	20194826* <sup>§</sup>
				Peripheral blood	Increased <i>BDNF</i> methylation was significantly associated with SI and depression in female patients 1 year after breast surgery and this association was independent of previous depression, and <i>BDNF</i> genotype. No significant methylation–genotype interactions were found	25838322*
				Peripheral blood	Higher <i>BDNF</i> DNA methylation levels significantly associated with previous SA, SI during antidepressant treatment, and SI at the last treatment session	23992681* <sup>§</sup>
				Peripheral blood	Higher DNA methylation at <i>BDNF</i> promoter is significantly associated with SI at baseline in a geriatric Korean population	24731781* <sup>§</sup>

<i>ARG2</i>	arginase 2	Suggested role in nitric oxide and polyamine metabolism	Role in the regulation of extra-urea cycle, arginine metabolism and down-regulation of nitric oxide synthesis	BA 44	DNA methylation in the promoter region of <i>ARG2</i> showed a relationship to the respective levels of expression in suicides	22008221*
				BA 44	Increased expression of <i>ARG2</i> in suicide completers correlates with decreased DNA methylation levels of specific CpGs in the promoter region	23260169*
<i>AMD1</i>	S-adenosylmethionine decarboxylase	polyamine biosynthesis	Polyamines biosynthesis	BA 44	Site-specific DNA methylation in the promoter region of <i>ADM1</i> was associated with <i>ADM1</i> gene expression in suicide cases compared to controls	22008221*
				BA 44	Increases in gene expression of <i>AMD1</i> in suicide completers correlate with decreases in methylation of specific CpGs in the promoter of this gene	23260169*
<i>GRIK2</i>	glutamate receptor ionotropic kainate 2	Regulation of circuit activity in neuronal cells, through G-protein coupled receptor stimulation	Ion channel, receptor activity	PFC (BA 8/9, BA10)	<i>GRIK2</i> is hypomethylated in suicide cases compared to controls. <i>GRIK2</i> showed higher levels of expression in cases	24662927* <sup>§</sup>
<i>BEGAIN</i>	brain-enriched guanylate kinase associated protein	Structural role in the postsynaptic density	Transmission across chemical synapses and protein-protein interactions at synapses	PFC (BA 8/9, BA10)	<i>BEGAIN</i> is hypomethylated in suicide cases compared to controls. <i>BEGAIN</i> showed higher levels of expression in cases relative to controls	24662927* <sup>§</sup>

<i>SKA2</i>	spindle and kinetochore associated complex subunit 2	Structural role, microtubule organization. Potential role in suppressing cortisol following stress	microtubule binding (Cell cycle, Cell division, Mitosis)	FC	DNA hypermethylation at C allele of rs7208505 predicted lower <i>SKA2</i> expression in suicide completers	25073599* <sup>§</sup>
				Blood and saliva	DNA methylation variation at <i>SKA2</i> gene mediates vulnerability to SB and PTSD through dysregulation of the HPA axis in response to stress	26305478* <sup>§</sup>
<i>ELOVL5</i>	Elongation of very long chain fatty acids protein 5	Elongation of long-chain polyunsaturated fatty acids	Polyunsaturated fatty acid biosynthesis	Plasma	SA significantly associated with DNA methylation in <i>ELOVL5</i> gene regulatory regions	25972837* <sup>§</sup>
<i>GAL, GALR3</i>	amino acid neuropeptide galanin, galanin receptor 3	Mood regulation	Chemical synaptic transmission, inflammatory response, nervous system development	LC, DRN	Increased <i>GAL</i> and <i>GALR3</i> mRNA levels, in parallel with decreased DNA methylation suicide cases compared with controls	27940914*
				BA 8/9, BA 24	<i>GAL</i> and <i>GALR3</i> transcript levels were decreased, <i>GALR1</i> was increased, and DNA methylation was increased in the DLPFC of male suicide cases compared to controls	27940914*
<i>TNF-A</i>	Tumor necrosis factor alfa	Cell signalling protein (cytokine)	Pro-inflammatory cytokine involved in inflammatory and immune responses, mediating neuronal death in injured brain	DLPFC	<i>TNF-A</i> promoter significantly hypomethylated in the suicide cases compared with the control group	29361849
<i>MARCKS</i>	Protein Kinase C Substrate	Metabolism and Integration of energy metabolism	calmodulin binding and protein kinase C binding	DLPFC	DNA hypomethylation (cg24011531) in the <i>MARCKS</i> gene in violent suicide cases compared to controls	29361849

<i>PSORS1C3</i>	Long non-coding RNA	Long non-coding RNA with a postulated role in immune system regulation	not reported	PFC (BA 11 and 25)	Significantly hypomethylated DMRs upstream of the <i>PSORS1C3</i> gene in both cortical regions in depressed suicide cases compared to controls	28045465* <sup>§</sup>
<b>Histone modifications</b>						
<i>OAZ1</i>	Ornithine antizym decarboxylase e 1	Role in cell growth and proliferation by regulating intracellular polyamine levels	intracellular polyamine biosynthesis and uptake	VLPFC (BA44)	Increased H3K4me3 levels in the promoter region of <i>OAZ1</i> in suicide completers correlated with the expression of <i>OAZ1</i> and <i>ARG2</i>	22008221*
<i>NTRK2</i>	Neurotrophic Tyrosine Kinase Receptor Type 2	Receptors of neurotrophins	Role in neurotransmission, calcium release, synaptic plasticity, and cell survival	PFC (BA10)	Increased methylation at H3 Lysine 27 in suicide brains compared to controls. Significant correlation between increased H3 lysine 27 methylation and TrkB.T1 expression level	19696771
<i>SYN1, SYN2 and SYN3</i>	Neuronal phosphoproteins	Synaptogenesis, synaptic transmission and synaptic plasticity	Regulation of axonogenesis and synaptogenesis	PFC (BA10)	H3K4me3 promoter enrichment in psychiatric suicide cases relative to controls	22571925* <sup>§</sup>
<i>CX30, CX43</i>	Connexin 30 and 43	Diffusion of ions and metabolites between the cytoplasm of adjacent cells	Gap junction channels	NEOCORTEX (BA4, BA17)	Enrichment of H3K9me3 for both <i>CX30</i> and <i>CX43</i> in the PFC of depressed suicide cases	27516431

**Abbreviations:** PFC, Prefrontal cortex; FPC, frontopolar cortex; DLPFC, Dorsolateral Prefrontal cortex; LC, Locus Coeruleus; DRN, dorsal raphe nucleus; SZ, Schizophrenia. \*Study used age matched cases and controls and/or included age as a co-variate in their analysis. <sup>§</sup> Study used gender matched cases and controls and/or included gender as a co-variate in their analysis.

**Table 2** Differentially expressed miRNAs associated with suicidal behavior

non-coding RNA	PUTATIVE / VALIDATED TARGET GENE	TARGET GENE FUNCTION (NCBI)	TISSUE	KEY FINDINGS	REFERENCES (PMID)
<b>MicroRNAs</b>					
miR-152 miR-181a miR-330-3p miR-34a	NA	NA	PFC (BA10)	8 miRNAs were significantly altered (2 up, 6 down) in psychiatric suicide subjects	24475125* <sup>§</sup>
miR-34c-5p miR-320c	<i>SAT1</i>	Enzyme in polyamine catabolism	PFC (BA44)	miRNAs targeting the 3' UTR of <i>SAT1</i> upregulated in PFC of suicide cases and lower expression levels of <i>SAT1</i> gene in suicide cases vs controls	24025154* <sup>§</sup>
miR-139-5p miR-320c	<i>SMOX</i>	Enzyme in polyamine catabolism (Roles in neurotransmission through the regulation of cell-surface receptor activity, involvement in intracellular signalling pathways)	PFC (BA44)	miRNAs targeting the 3' UTR of <i>SMOX</i> upregulated in PFC associated to a lower expression levels of <i>SMOX</i> gene in suicide cases	24025154* <sup>§</sup>
miR-20b miR-20a miR-34a miR- 34b	<i>BCL2</i> <i>DNMT3B</i> <i>MYCN</i> <i>VEGFA</i>	cellular growth and differentiation	PFC (BA9)	Globally downregulated expression pattern of miRNAs in the PFC of depressed suicide subjects	22427989
miR-34b–5p miR-369–3p	<i>NOTCH</i>	neuronal plasticity (sequence-specific DNA binding transcriptional activator activity, RNA polymerase II transcription factor binding)	Peripheral blood	Expression levels of miR-34b–5p and miR-369–3p were significantly lower in leucocytes of Chinese MDD	26807671* <sup>§</sup>

miR-185	<i>NTRK2</i>	Receptors of neurotrophins Neurotransmission, calcium release, synaptic plasticity and cell survival	PFC (BA10)	The miRNA miR-185 found differentially expressed and inversely correlated with TrkB-T1 expression in the PFC of suicide completers	22802923
miR-185 miR-195 miR-30a miR-49	<i>DICER1</i> <i>NTRK2</i>	miRNA bio-synthesis (nucleic acid binding and hydrolase activity)	PFC	<i>DICER1</i> expression level associated with SB in both MDD and BD samples; Polygenic profile scores negatively predicted SB in the BP sample for only 4 miRNA genes	26921221
miR-19a-3p	<i>TNF-A</i>	mediating neuronal death in injured brain	DLPFC	Specifically upregulated in individuals who died by suicide compared to normal controls	29361849
<b>Long non-coding RNAs</b>					
LOC285758	<i>MARCKS</i>	Metabolism and Integration of energy metabolism (calmodulin binding and protein kinase C binding)	DLPFC	<i>LOC285758</i> expression is significantly increased in violent suicides irrespective of <i>MARCKS</i> transcription levels but not the opposite	24821221* <sup>§</sup>
TCONS_00019174 ENST00000566208 NONHSAG045500 ENST00000517573	NA	Protein complex biogenesis	PBMCs	Expression of six down-regulated lncRNAs had a negative association with suicide risk in MDD patients	28638716

**Abbreviations:** PFC, Prefrontal cortex; DLPFC, Dorsolateral Prefrontal cortex; LC, Locus Coeruleus; DRN, dorsal raphe nucleus; PBMCs, peripheral blood mononuclear cells; MDD, major depressive disorder; BD, bipolar disorder. \*Study used age matched cases and controls and/or included age as a co-variate in their analysis. <sup>§</sup> Study used gender matched cases and controls and/or included gender as a co-variate in their analysis.