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Transcriptomic and Epigenomic Biomarkers of Antidepressant Response

Raoul Belzeaux[1], Rixing Lin[1], Chelsey Ju[1], Marc-Aurele Chay[1], Laura M. Fiori[1], Pierre-Eric Lutz[1,2], Gustavo Turecki[1]

[1]McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, Montreal, Quebec, Canada.

[2] Institute of Cellular and Integrative Neuroscience, CNRS, UPR3212, Strasbourg, France

Highlights

- Antidepressant treatment is associated with a high rate of poor response
- Our review highlights the challenges to develop biomarkers of antidepressant response
- Several mRNAs and micro-RNAs have been described as potential biomarkers but replications are needed
- DNA methylation and histone modifications are also a promising avenue to develop biomarkers

Abstract

Background

Antidepressant treatment is associated with a high rate of poor response, and thus, biomarker development is warranted.

Methods

We aimed to synthesize studies investigating gene expression, small RNAs, and epigenomic biomarkers of antidepressant response. We conducted a narrative review of the literature.

Results

Firstly, we detailed the challenges involved, in terms of biological tissues, relevant study time frames, and mandatory statistical tools. Secondly we synthesized results obtained in gene expression studies, focusing mainly on genome-wide studies, particularly small non-coding RNA, including micro-RNA and other small RNA species. In addition, we reviewed the potential biomarkers of antidepressant response arising from studies investigating DNA methylation variation and histone modifications.

Limitations

We did not conduct a meta-analysis due to the heterogeneity of the study.

Conclusion

Although promising, the field of gene expression and epigenomic biomarkers of antidepressant response is still in its infancy, and needs further development to define useful biomarkers in clinical practice.

According to practical guidelines, antidepressants are the first-line treatment for moderate to severe major depressive episodes (MDE) (Kennedy et al., 2016). However, a significant proportion of individuals treated with antidepressant treatment do not respond or remit. Indeed, 40% of patients fail to achieve remission after four different antidepressant treatments (Rush et al., 2006). It has become increasingly clear that major depression is a heterogeneous illness, and that antidepressant response, while currently unpredictable, is not a random outcome. Antidepressant response can be considered to be a complex phenotype, with individual variability resulting from an interplay between clinical factors and biological pathways, as well as gene x environment interactions (El-Hage et al., 2013). However, to this date, no specific socio-demographic or clinical markers have proven successful for prediction of antidepressant response (Gadad et al., 2017). As a consequence, biomarkers that demonstrate high accuracy in predicting antidepressant response would be invaluable. According to the American Food and Drug Administration (FDA), a biomarker is a defined characteristic that is measured as an indicator of normal or abnormal biological processes, or the response/resistance to an exposure or intervention including pharmacological treatment (Group, 2016). A biomarker may be molecular, biochemical, radiological, etc.

In this review we aimed to synthesize studies investigating transcriptomic, including small RNA species, and epigenomic markers of antidepressant response. We focused on these molecular biomarkers because they represent products and/or biological mechanisms of genes and their interaction with the environment. Firstly, we reviewed the challenges associated with the development of biomarkers in this field. Secondly, we conducted a narrative review of available data that describes potential epigenomic / transcriptomic biomarkers of antidepressant response. We concentrated our review on data obtained in living human subjects and studies that focused specifically on antidepressant response; for areas where few or no human studies have been published, relevant animal or cellular studies are briefly described to provide evidence for future studies. We used Pubmed to conduct the literature review, with the following key word combinations: “antidepressant response”, “gene expression”, “mRNA”, “miRNA”, “epigenetic regulation”, “methylation” and/or “histone modification”. We included in this review only the most significant studies based on the originality of the study, impact of the findings and sample size. We described and discussed results from studies identified, and did not pool data.

1) Challenges

    a. Where? Overpassing the "brain-blood barrier"

The first challenge when developing a biomarker of treatment response based on gene expression and epigenomic markers is the selection of tissue in which to measure this biomarker. Biological fluids are the most practical tissues for patient sampling, particularly peripheral blood samples, as they are easy and quick to retrieve from multiple patients. Saliva, buccal and skin cells, along with cerebrospinal fluid, have been noted as possible tissue sample sources, but caveats are present in each case (Levenson, 2010) and, as a consequence, the vast majority of proteomic/genomic/epigenomic markers have been described in blood. It is worth noting that saliva is easy to collect. It has been suggested that saliva methylation patterns are closer to those of brain tissue than other peripheral tissues (Langie et al., 2016), but to what extent acquired methylation changes in genomic loci relevant to depressive psychopathology or treatment response may be detected in saliva as proxy to those taking place in the CNS remains unclear. In addition, mRNA and protein studies are challenging in saliva. Moreover, bacterial DNA contamination can bias the results. Skin cells are also an interesting tissue, but more frequently used for cell culture. Variations related to clinical state and treatment response remain to be demonstrated. Cerebrospinal fluid is interesting due to its close relationship with brain physiology but hard to collect and, in physiological conditions, contains few cells and circulating proteins. Alternatively, whole blood is easily accessible in patients before and during treatment, without major ethical considerations. Moreover, blood samples allow simultaneously investigation of several different biomarkers (i.e. DNA polymorphisms, DNA methylation and other epigenetic markers, all RNA species, and proteins) using the same sample. In addition the investigation of blood samples provide high concentrations and quality of DNA and RNA, and allow for cells to be cultured for functional studies. It is worth noting that blood samples also have significant limitations. Blood contains several cell types, and relative differences in cell compositions between groups may lead to biases in case-control studies. In addition, the relationship between genomic, epigenomic and proteomic peripheral changes measured in blood to those in the CNS may not always be relevant. However, considering that depression is, to some degree, a systemic illness, and factors influencing treatment response include systemic factors, blood samples may provide meaningful insight into mechanisms related to antidepressant response.

Although the primary pathophysiological mechanisms of MDE and antidepressant responses are based in the brain, depression is a systemic illness with symptoms affecting multiple organs and systems. Not surprisingly, several lines of evidence suggest that there is validity to studying certain biomarkers of MDE in peripheral tissues.

i. MDE and antidepressant response involves not only brain / CNS systems: Two common hypotheses regarding major depression are based on stress regulation and monoaminergic neurotransmitters. Stress regulation largely revolves around the hypothalamic-pituitary-adrenal (HPA) axis, which impacts multiple organs and tissues besides the brain. Corticosteroids are produced by the adrenal gland and excreted into circulation to bind glucocorticoid receptors (GR) and mineralocorticoid receptors (MR), which are expressed in all tissues (Anacker et al., 2011). Newer evidence suggests that immune dysregulation (Raison et al., 2006) as well as the gut microbiome may also be implicated in the pathophysiology of depression (Foster and McVey Neufeld, 2013). Pro-depressant effect of immunomodulators has been described (e.g., Interferon-α). This is further supported by evidence demonstrating anti-inflammatory and/or immune effects of antidepressants (Eyre et al., 2016).
ii. Several antidepressant targets are expressed in other tissues: a number of serotonin receptor subtypes are expressed in blood vessels and/or immune cells, and the serotonin transporter (SLC6A4) is expressed in platelets and in some immune cells (Gladkevich et al., 2004).
iii. The immune system and nervous systems share several neurochemical pathways and the expression level of numerous genes appear to be correlated between blood and brain regions, in particular the pre-frontal cortex and the amygdala (Sullivan et al., 2006).
iv. Many RNA molecules, in particular micro-RNA (miRNA), circulate in cell-free fluids, and it is possible that they could act distally, such as hormones.

b. When? Phenotypic and clinical challenges

Another challenge when describing biomarkers is to determine the time frame of study and time of sample collection. These choices are driven by the nature of the biomarker. Both predictive and moderator biomarkers can be used to predict the outcome, such as antidepressant response, prior to commencing treatment. Predictive biomarkers can be used without taking into account different conditions, i.e. predict clinical improvement in patients whatever the treatment proposed. Alternatively, moderator biomarkers can be used to predict an outcome in a specific condition, i.e. to predict clinical improvement in patients receiving a specific antidepressant in comparison to another treatment strategy or a placebo. Although predictive biomarkers are interesting, moderator biomarkers are more clinically relevant to improve patients' care. Indeed, moderator biomarkers would allow clinicians to choose the best option between available treatments, whereas predictive biomarkers would only indicate overall prognosis. The majority of previous studies described only predictive biomarkers. However, newer studies by our group and others have now included a placebo group and/or different antidepressant treatments, which allow for the identification of moderator biomarkers (Lam et al., 2016).

It must be noted that the identification of predictive or moderator biomarkers ideally require tissue collection and data acquisition before treatment initiation. However, it is uncommon for studies to include antidepressant naïve patients as they typically have already been treated by a general practitioner or first line psychiatrist prior to study recruitment. To overcome this issue, patients are often required to cease treatment with medications that are considered as ineffective for at least five drug half-lives, before inclusion in the study. From a pharmacological point of view, this allows the elimination of the antidepressant from the body. However, it is uncertain if its biological effects will be totally reversed within this time frame, particularly as transcriptomic and epigenetic effects induced by antidepressants may have different, and longer, kinetics.

Biomarkers can be described as biological variation occurring during response, and can be used to monitor treatment response. Mediator biomarkers, also referred to as target biomarkers (Cattaneo et al., 2013), describe within-subject variations across time. The majority of antidepressant studies to-date has evaluated patients at two time points: at baseline (just prior to treatment), and at an end-point (typically after 6 to 8 weeks of treatment). Differences in clinical

scores between these two time points is used to differentiate responders from non-responders, i.e. patients showing an > 50% improvement from baseline depression severity. However, additional measurements at other time points could be interesting, particularly to identify early biological changes and clinical improvement (first two weeks), which may have a predictive value for later antidepressant response (Leuchter et al., 2010).

Lastly and independently of the nature of the biomarker, numerous additional factors may impact interpretation of results. The time of day at which samples were collected may be relevant, as mood disorders have been associated with alterations of circadian rhythms (Li et al., 2013). Similarly, factors such as age, sex, smoking, diet, pollution, and drug use may also affect findings.

c. How: statistical tools and other methodological challenges

It is worth noting that a biomarker for antidepressant response should not be defined simply by a statistical difference between responders and non-responders. Beyond this first step, the accuracy of a biomarker (i.e. clinical validity, or ability to distinguish between groups) is an important characteristic that can be assessed using Receiver Operating Characteristic (ROC) curves analysis, logistic regression or machine learning approaches. Moreover, clinical utility, medico-economic validity and biological plausibility are also important issues to examine when describing a new biomarker of antidepressant response. We recently reviewed these steps elsewhere (Belzeaux et al., 2017b).

2) Possible biomarkers involved in gene expression and epigenetic regulation

Gene expression regulation involves a complex interaction between several biological mechanisms. Different RNA species have been described according to their function and size. Figure 1 describes processes involved in the regulation of gene expression that may harbour biomarkers for psychiatric disorders in general, and more specifically for antidepressant response.

Messenger RNAs (mRNA) are transcribed in the nucleus, then exported to the cytoplasm where they are translated to proteins. Transcription is highly regulated by reversible changes in DNA

conformation and compaction, most commonly through methylation of DNA and post-translational modification of histone tails. The use of alternative promoter regions (i.e, starting point of transcription) and alternative splicing (i.e. inclusion or exclusion of particular exons) add complexity to the transcriptome. Each of these mechanisms shows developmental- and tissue-specific dependency.

In addition to mRNA, other forms of RNA are defined as "non-coding" and classified according to size and function. They act as regulators of transcription, translation, or mRNA stability, as described below and in Figure 1. We focus this review on mRNA, several classes of small non-coding RNA, DNA methylation, and histone modifications as potential biomarkers of antidepressant response.

3) Review of studies to-date

a. Messenger RNA (mRNA) based on candidate biomarkers

Many gene expression studies focusing on protein coding gene expression have been conducted with the aim of identifying biomarkers for antidepressant response. Protein coding genes are well-suited for hypothesis-driven studies based upon current knowledge regarding the pathophysiology of MDE as well as the molecular mechanisms of antidepressants. Unfortunately, none of these hypothesis-driven studies have identified robust biomarkers of antidepressant response, and their findings have been poorly replicated. The serotonin transporter (SLC6A4) is the main target of numerous antidepressants and has been identified as a potential candidate biomarker involved in antidepressant response, although genetic studies have found conflicting results (Gadad et al., 2017). Numerous studies based on relatively small cohorts described variation of the serotonin transporter gene peripheral expression according to depressive symptoms of patients compared to psychiatrically healthy controls (Belzeaux et al., 2010; Belzeaux et al., 2014b; Iga et al., 2005; Lima et al., 2005; Tsao et al., 2006). Previous studies demonstrated that SLC6A4 mRNA could vary according to the emergence and the treatment of MDE (Belzeaux et al., 2014a). Moreover, it seems that its expression appears to be stable over a 30-week period in healthy controls (Belzeaux et al., 2014b). As a consequence,

SLC6A4 mRNA may be an interesting mediator biomarker of antidepressant response, but more studies in larger cohorts are needed.

Based on the hypothesis of an involvement of low grade inflammation in antidepressant resistance, previous studies that investigated Interleukin 1 β (IL1β) found that: (i) it is over-expressed before treatment in non-responders (Cattaneo et al., 2016; Cattaneo et al., 2013) and that (ii) antidepressant treatment reduced its expression in response to an immune (LPS) stimulation (Himmerich et al., 2010). These results suggest that IL1β mRNA levels are a potential predictive and mediator biomarker. In the same vain, based on pharmacogenomics findings of association between IL11 genetic variants and antidepressant response, one study have reported variation in IL11 expression according to antidepressant response (Powell et al., 2013a). In the same study, the authors demonstrated that the level of expression of the pro-inflammatory cytokine tumor necrosis factor (TNF) was predictive of antidepressant response (Powell et al., 2013a). Again, more studies are needed to better quantify the accuracy of these biomarkers, and to replicate these findings in an independent cohort.

Finally, FK506 Biding Protein 5 (FKBP5 or FKBP-51), a co-chaperone protein involved in glucocorticoid response, has been investigated in several studies. A number of genetic studies have demonstrated an association between single nucleotide polymorphisms in FKBP5 and antidepressant response (Gadad et al., 2017). Interestingly, this protein has been identified as a regulator of the epigenetic regulation of Brain-Derived Neurotrophic Factor (BDNF) according to antidepressant treatment and response (Gassen et al., 2015). It has been shown that antidepressant treatment decreases FKBP5 expression only in responders, suggesting that this mRNA could be an interesting mediator of antidepressant response (Cattaneo et al., 2013). Although this result needs to be replicated, FKBP5 shows potential as a biomarker of antidepressant response with good biological plausibility.

b. Messenger RNA (mRNA) based on whole transcriptome analyses

In addition to hypothesis-driven approaches, numerous hypothesis-free, genome-wide studies, such as those using microarrays and next-generation sequencing, have been published. These allow for the simultaneous measurement of the expression of thousands of transcripts: before treatment, to define predictive biomarkers; and during treatment, to define mediators. Through

this approach, new candidate loci and biological pathways can be identified. It should be noted that this approach is associated with an increase in type 1 errors due to multiple testing. As a consequence, studies that properly control for this type of error, and use multilevel strategies to investigate the technical reproducibility and biological plausibility of their findings, are more likely to produce scientifically valid results.

One challenge when analysing large data sets is to determine the best strategy to define the best list of candidate molecules. Unbiased machine learning model approaches can allow the selection of the best combination of molecular markers to predict antidepressant response or remission as proposed by Guilloux et al. (Guilloux et al., 2015). In this work, the authors described a set of 13 genes that allow the prediction of remission (i.e. absence of symptoms after treatment) upon treatment with citalopram, with an accuracy of 79.4%. Interestingly, they replicated their findings in an independent cohort and found a corrected accuracy of 76%. Based on a small sub-cohort of the Munich Antidepressant Response Signature, another study described several mRNAs as potential predictors of antidepressant response in a naturalistic study design (i.e. patients were treated according to their psychiatrist's choice) (Hennings et al., 2015). Interestingly, they replicated their findings in a larger cohort, highlighting three new potential predictive biomarkers of treatment response as well as remission, i.e. RAR related orphan receptor A (RORa), germinal center associated signaling and motility (GCET2) and SWI/SNF related, matrix associated, actin dependent regulator of chromatin subfamily c member 2 (SMARCC2). RORa was of particular interest due to its implication in response to cellular stress and circadian rhythms. Two additional studies (Mamdani et al., 2014; Pettai et al., 2016) also identified potential predictor biomarkers, but their results have not been replicated in independent samples. Among the predictor or mediator biomarkers which have been described, the majority display relatively low biological plausibility, largely resulting from lack of knowledge regarding the cellular function of their protein products in brain tissue (Pettai et al., 2016). Lastly, in a small microarray study, Belzeaux et al. demonstrated that IL1β and TNF (see Table 1), as well as two other genes not previously implicated in psychiatric disorders (i.e. histone cluster 1 H1 family member e (HIST1H1E) and palmitoyl-protein thioesterase 1 (PPT1)) could be selected as best predictive biomarkers without a priori hypotheses (Belzeaux et al., 2012). In this study, the authors used a combination of the best predictive mRNA and described

the predictive value of this combination using ROC curve analysis. Overall, the majority of these findings remain to be replicated by independent studies.

A few studies, including some of those listed above, have focused their analysis on identifying mediator biomarkers by analysing gene expression changes before and after antidepressant treatment using microarrays (Hennings et al., 2015; Hodgson et al., 2016; Mamdani et al., 2011; Pettai et al., 2016). Our group demonstrated that interleukin regulatory factor 7 (IRF7) may be an interesting mediator biomarker of citalopram response, and also demonstrated low expression of this gene in *post-mortem* brains of depressed suicide samples (Mamdani et al., 2011). Another group conducted a comparable study with patients treated with escitalopram or nortriptyline (Hodgson et al., 2016). They were not able to detect a mediator of antidepressant response in the entire sample or in patients treated with escitalopram. However, they demonstrated that responders treated with nortriptyline had a specific dysregulation of two genes, matrix metallopeptidase 28 (MMP28) and KxDL motif containing 1 (KXD1).

c. Gene co-expression networks

mRNA expression studies provide further information for exploring the biological processes involved in antidepressant response and MDE through network analyses of gene expression. Network analysis provides new opportunities when exploring gene expression changes or differences according to a specific phenotype. Firstly, particularly in cases when phenotypes are associated with numerous changes with small effect sizes, as in psychiatric disorders, the use of gene co-expression modules (i.e. sets of gene displaying correlated expression) provides an interesting strategy for data mining and reduces the impact of multiple testing strategies. While such correction for multiple testing is mandatory to reduce false positive findings, it also associates with an increase in false negative findings and decreases the ability to replicate findings between studies. Moreover, gene network exploration represents an opportunity to capture emergent properties, i.e. characteristics of a network that are not predictable by the sum of the characteristics of each part of the network. In other words, emergent properties could be a new organisation of the transcriptome associated with a phenotype or a treatment. Lastly, this type of analysis provides interesting insight into biological pathways related to each module. Genes that are part of the same module are “guilt by association” and one could interpret that

they are related to a specific biological function (Gaiteri et al., 2014). As a consequence, biological pathways associated with specific modules may be more biologically relevant to a phenotype of interest than a pathway analysis of genes sets based on differential expression between groups. Moreover, one module may be regulated by a "meta-regulator" such as a miRNA, an enhancer or re-organization of chromatin structure. For example, correlated expression of genes may be due to their regulation by a small set of common miRNAs (see below). To the best of our knowledge, only two studies have proposed this approach in the field of antidepressant response, known as the weighted correlation network analysis (Langfelder and Horvath, 2008): one based on a predictor approach (Belzeaux et al., 2016), and one based on a mediator approach (Hodgson et al., 2016). In the first study, this method was used to replicate the potential modules associated with treatment response between three different cohorts (Belzeaux et al., 2016), and found an interesting overlap of findings across studies, particularly for modules related to immune response, acute inflammatory response and C-X-C motif chemokine ligand 8 (IL8) receptor activity. This study also demonstrated that modules associated with antidepressant response are not specifically enriched for differentially expressed genes, i.e. that significant differential expression between responders and non-responders at gene level is not mandatory for a biological process to be associated with antidepressant response. This result suggests that antidepressant response is associated with emergent property of the gene co-expression network. Finally, genes of modules associated with antidepressant response were predicted to be highly regulated by common miRNAs. In other words, using bio-informatic tools, findings suggested that miRNA may be a major meta-regulator of antidepressant response, reinforcing the interest in other RNA species in the field of biomarkers of antidepressant response.

d. Micro RNA (miRNA)

Small non-coding RNAs (sncRNAs) have recently gained attention in the field of biomarker discovery (Issler and Chen, 2015). A growing number of studies have suggested that miRNAs may be excellent candidates as biomarkers. MiRNAs are single-stranded non-coding RNAs that are 17 to 22 nucleotides long and typically found associated with the AGO family proteins to form the RNA-induced silencing complex (RISC) (Ha and Kim, 2014; Wahid et al., 2010).

RISCs act by inducing degradation or translational silencing of their mRNA target. Target recognition is primarily determined by complementarity between a stretch of 6 nucleotides near the 5'-end of the miRNA (seed region) and the 3'UTR region of its target mRNA (Wahid et al., 2010). miRNAs represent an important class of gene-regulatory molecules, and thus dysregulation of miRNA expression is believed to play a major role in disease etiology. This has been suggested for depression based on *post-mortem* studies and animal studies (Belzeaux et al., 2017b). Moreover, the focus on miRNAs in psychiatry is reinforced, in part, by the discovery that they are released by cells in small vesicles known as by exosomes and circulate in peripheral blood (Quek et al., 2017; Valadi et al., 2007). As such, it is possible that circulating miRNAs in peripheral blood may reflect miRNA expression/dysfunction in the brain or other tissue types.

The investigation of miRNA in depression has been growing rapidly and several studies have identified associations between miRNA and MDE or antidepressant response. We recently reviewed evidence and future perspectives in biomarker development based on miRNA studies (Belzeaux et al., 2017b). We found that, to this date, few miRNAs have been strongly associated with antidepressant response. Among them, based on a systematic literature review, we selected miR-1202, miR-124, miR-135a, miR-145 and miR-20b as best candidates (Belzeaux et al., 2017b). However, the predictive value and the development of these miRNA as biomarkers need further study. Lastly, a very recent study by our group demonstrated translational evidence that miR-146a-5p, miR-146b-5p, miR-425-3p and miR-24-3p expression levels were decreasing during response with antidepressant treatment. As a consequence, these four miRNAs could be mediator biomarkers of antidepressant response (Lopez et al., 2017). The results were replicated in two independent cohorts of patients treated with different antidepressants, as well as in a well-characterized animal model of MDE (i.e. mice susceptible to chronic social defeat, treated with imipramine). Moreover, we demonstrated that these miRNAs were over-expressed in ventrolateral prefrontal cortex tissue of depressed patients, and were predicted to target genes enriched within the MAPK/Wnt pathways. To the best of our knowledge, this encouraging study is the only sufficiently powered translational study conducted to describe miRNAs as mediators of antidepressant response. These results, if confirmed by other groups, may lead to the development of relevant biomarkers to monitor antidepressant response. Moreover, these mediator biomarkers could lead to a better understanding of the mechanisms of antidepressant response.

e. Other small non-coding RNA (sncRNA)

Other sncRNAs have been less well investigated in psychiatry. Although this group comprises a large number of different RNA species, the most suited for biomarker discovery include small nucleolar RNA (snoRNA), small nuclear RNA (snRNA), and PIWI (P-element induced wimpy testis)-interacting RNA (piRNA), as they exhibit robust expression in peripheral blood (Freedman et al., 2016) and their biological roles and mechanisms have been better established (Falaleeva et al., 2015; Iwasaki et al., 2015; Kishore and Stamm, 2006; Lee et al., 2011; Lui and Lowe, 2013; Rajasethupathy et al., 2012). SncRNA have protein-binding partners and guide these proteins to target RNAs via sncRNA-target RNA sequence complementarity. Results from these interactions vary from sncRNA to sncRNA. sncRNA called snoRNA can guide chemical modifications on target RNAS, while snRNA can affect splicing of target pre-mRNA (see Figure 1).

To date, there are no publications investigating other sncRNAs in MDE and antidepressant response. However, there is evidence demonstrating that snoRNAs (Falaleeva et al., 2015; Kishore and Stamm, 2006) and piRNAs (Lee et al., 2011; Rajasethupathy et al., 2012) have specific roles in the human central nervous system (CNS), making them potential candidates for MDE and/or antidepressant response.

f. DNA methylation

DNA methylation is a prominently studied epigenetic mark, associated with various cellular mechanisms such as X chromosome inactivation, imprinting, and chromatin remodeling (Curradi et al., 2002). From a clinical perspective, DNA methylation biomarkers have been implicated in several diseases, including various cancers (deVos et al., 2009; Laird, 2003), neurodevelopmental disorders (Robertson and Wolffe, 2000), and autoimmune diseases (Li, 2002).

Methylation refers to the presence of a methyl group in the 5th position of a cytosine to form 5'-methylcytosine (5mC), a reaction that is catalyzed by members of the DNA methyltransferase (DNMT) family. In mammals, DNA methylation predominantly occurs at cytosine positions

immediately followed by a guanine (CpG). Non-CpG methylation occurs at a much lower level, and it is primarily detected in the CNS. Its relevance to biomarker discovery remains to be determined. CG dinucleotides are less frequent than expected in the human genome. However, large clusters of CpGs, known as CpG "islands" (CGIs), are often unmethylated and found in most promoter regions (Mamrut et al., 2013; Maunakea et al., 2010b). More precisely, promoters enriched with CpG islands tend to be unmethylated, within housekeeping genes expressed throughout the organism. Conversely, promoters with less CpG content tend to be more methylated. These promoters are associated with genes with tissue-specific expression profile (Vinson and Chatterjee, 2012; Weber et al., 2007). Multiple studies show that active promoters are generally unmethylated, whereas inactive promoters tend to be methylated (Eckhardt et al., 2006) although there are exceptions. Collectively, these properties indicate that methylation at CpG sites has a significant role towards regulating transcription (see Figure 1), more often in a repressive manner (Vinson and Chatterjee, 2012). Methylation within promoter regions can directly affect gene expression by blocking transcription factors and their downstream activity (Pérez et al., 2012). Alternatively, promoter methylation can indirectly disturb gene expression by recruiting proteins with methylated-DNA binding domains (MBDs) that modify chromatin structure (Baylin and Jones, 2011; Curradi et al., 2002). The functional implication of DNA methylation in other genomic regions (i.e. gene bodies, intergenic region) while less well-characterized, tends to associate with increase patterns of expression (Maunakea et al., 2010a), which may or may not be explained by increased hydroxymethylcytocin in inragenic marks (Gross et al., 2015).

DNA methylation biomarkers are suitable for *in vitro* applications for multiple reasons, one of which is stability in biological samples. Methylation patterns have been found to be faithfully retained in samples after an extended period of storage (The, 2016). Multiple techniques have been developed for detection of methylation levels, many of which require treating DNA with sodium bisulfite, which converts unmethylated cytosines to uracil while methylated cytosines are unchanged, and then performing downstream molecular assays to detect DNA methylation frequency (Levenson, 2010). We recently reviewed the different technical approaches available (Fiori and Turecki, 2016). Despite ongoing improvements, multiple types of methylation assays are currently available to provide fast and robust quantification required for biomarker development (The, 2016).

A number of target-based studies have now been performed to investigate MDE and antidepressant response. The levels of BDNF have been repeatedly linked to antidepressant response, and lower methylation levels at CpG sites within the BDNF promoter were found to be associated with antidepressant response and decreased BDNF plasma levels after one week of treatment (Tadic et al., 2014). Interestingly, DNA methylation of BDNF has been described to be regulated by a biological cascade involving FKBP5 and DNMT1 according to antidepressant response (Gassen et al., 2015).

Hypermethylation of SLC6A4 was found in depressed patients displaying significant clinical responses after a 6-week treatment with escitalopram (Domschke et al., 2014), but another group who conducted a similar experiment with a 12-week treatment period did not find any reliable correlation between SLC6A4 methylation and antidepressant response (Kang et al., 2013). Moreover, baseline methylation at several CpG sites within the interleukin-11 (IL11) promoter were found to be predictive of escitalopram or nortriptyline treatment response rates after 12-weeks (Powell et al., 2013b). Finally, demethylation of Crh genes, involved in brain stress response, as been described in chronic social stress mice while resilient mice did not demonstrated such a change (Elliott et al., 2010).

Typically, studies investigating DNA methylation in blood have used either DNA extracted from whole blood lysates, or circulating leukocytes. One important consideration while investigating DNA methylation in blood is cellular composition, as factors that may influence cellular composition, such as inflammatory or infectious diseases, may be differentially distributed between groups. DNA methylation patterns are cell-type specific. As a consequence, differential cellular composition between groups would affect the methylation information obtained. When cell counts are not available, bioinformatic approaches have been developed to generate cell-type specific profiles ("deconvolution") from mixed samples.

As with the other molecular factors described in this review, DNA methylation can also be affected by additional socio-demographic and clinical variables (Brinkman et al., 2010; Li et al., 2012). In particular, early life adversity has been repeatedly associated with long-lasting changes in DNA methylation patterns in either peripheral tissues or brain-derived samples (Lutz and Turecki, 2014; Turecki, 2014). Studies have demonstrated that individuals abused during childhood are more likely to be diagnosed as adults with major depressive disorder, among others (Nemeroff, 2016), and a meta-analysis investigating the relationship between childhood

maltreatment and antidepressant treatment showed that abused participants were less likely to respond, or be in remission after a trial of antidepressants (Nanni et al., 2012). Additional socio-demographic factors that have been associated with differential methylation patterns include age, gender, smoking, and socio-economic status. Accordingly, biomarker discovery studies exploring DNA methylation should have experimental designs that assess and account for a variety of these potential confounders.

g. Histone modifications

i. Biology and mechanisms of histone modifications

Modification of histone tails, through the addition or removal of chemical groups, has significant impact on gene expression through effects on DNA compaction and protein recruitment at the site of transcription. Histone protein octamers make up nucleosomes, around which DNA wraps itself (Kornberg and Lorch, 1999). Depending on the type, location and combination of modifications present on a given histone tail, the physical properties of the chromatin are modified and contribute to the regulation of downstream transcriptional events (Bannister and Kouzarides, 2011). Histone acetylation, which is mediated by histone acetyltransferases (HATs) and removed by histone deacetylases (HDACs), typically associates with transcriptional activation (Brownell et al., 1996; Taunton et al., 1996). Methylation of histone residues, on the other hand, does not have a clear correlation with gene expression. Both lysine and arginine residues can be methylated by histone methyltransferases (HMTs), and demethylated through the action of demethylases (Allfrey et al., 1964; Kohli and Zhang, 2013). Examples of histone methylation include trimethylation of histone 3 lysine 4 (H3K4me3), associated with transcriptional initiation, and trimethylation of H3K27 (H3K27me3), associated with polycomb repressed genes (Rothbart and Strahl, 2014). Many other types of histone post-translational modifications exist, such as ubiquitination, sumoylation, and phosphorylation. The many possible combinations of modifications at various residues of histone tails make up the "histone code", which contributes to determining the epigenetic landscape of our cells (Jenuwein and Allis, 2001).

ii. Histone modifications and antidepressant response

Several studies using animal models have implicated histone modification in stress and/or depressive phenotypes, and a reversal of these effects following antidepressant treatment. In a study of chronic social defeat stress, Tsankova et al found a long lasting downregulation of *Bdnf* splice variants III and IV in the hippocampus, accompanied by an increase in repressive histone methylation at their promoters. Chronic imipramine treatment reversed this downregulation, and caused increased histone acetylation at these promoters (Tsankova et al., 2006). In the same vein, Chen et al evaluated the effects of antidepressants on human *post-mortem* prefrontal cortex, and found increased expression of BDNF IV which was associated with decrease in H3K27me3 at the promoter region (Chen et al., 2011).

Based on these modifications in animal and human *post-mortem* samples, and on the hypothesis that these results could be translatable to peripheral blood, histone modifications could be of significant interest for the discovery of biomarkers in antidepressant response. However, to this date, due to the large number of histone post-translational modification types, and the difficulty of reliably identifying changes on a genome-wide level, few studies have been conducted to assess their potential in predicting antidepressant response. A study of treatment-naïve MDE patients investigated peripheral blood H3K27me3 levels between citalopram responder and non-responders (Lopez et al., 2013). Patients who responded to the 8-week treatment were found to have a significant decrease in H3K27me3 at the promoter IV of the BDNF gene, and a concomitant increase in BDNF mRNA expression. These changes were not found in the non-responder group (Lopez et al., 2013), indicating that BDNF promoter H3K27me3 levels could serve as a potential biomarker for citalopram response in MDE patients.

Moreover, based on the same hypothesis several groups have characterized the variation of expression of histone modifying enzymes in peripheral blood. One study compared gene expression in leukocytes of control subjects and depressed patients, before and after paroxetine treatment during 8 weeks. Results showed that baseline levels of histone deacetylase 5 (HDAC5), was significantly higher in drug-free depressed patients, while they were normalized after paroxetine treatment (Iga et al., 2007). Multiple studies have been conducted comparing histone writer expression levels in blood of patients in a depressive vs remissive state. Comparison of expression levels of 11 HDACs found significant increases in HDAC2 and HDAC5 mRNA expression levels in depressive patients but not in patients in remission (Hobara et al., 2010). These effects, which were not found to be specific to any single antidepressant

treatment, suggest that patients who show depressive symptoms have a different histone modifying enzyme expression. As a consequence, these results suggest, indirectly, differences in the histone modification landscape in patients with depressive symptoms when compared to patients in remission. As such, binding sites of HDAC2 and HDAC5 should be investigated for potential biomarkers of antidepressant response.

A similar study interrogated the roles of sirtuins (SIRT, class III HDACs) in depressive states in peripheral white blood cells. SIRT1, 2 and 6 mRNA levels were significantly decreased in patients who were in a depressive state, but not in control or patients in remission (Abe et al., 2011).

4) Conclusions and perspectives

RNA and epigenetic biomarkers of antidepressant response define a promising area for the development of new biological tools in psychiatry. We synthesized in this review the challenges of such research and gave an overview of studies already published in this field. Of note, we conducted a narrative review and as such, it is possible, although unlikely, that we did not include all relevant papers in this report.

In this review, we did not address other areas of biomarker development in antidepressant response, in particular genetic variation and proteomic biomarkers. However, we have briefly cited some of this work when findings overlapped with those from studies reviewed here. Although the field of biomarker development has been active for more than 20 years, results have yet to support clinical application (Fabbri et al., 2013; Gadad et al., 2017). Importantly, as of yet, no medico-economic analyses have found there to be an unequivocal cost-effectiveness of this type of biomarkers (Verbelen et al., 2017).

Proteomics may also provide interesting biomarkers of antidepressant response, both in hypothesis-driven and hypothesis-free approaches. Limitations of multiplex tools and platforms as well as other more specific techniques have been described elsewhere (Belzeaux et al., 2017a; Gadad et al., 2017). Numerous proteomic biomarkers or combinations of biomarkers have been described but are beyond the scope of our review.

Finally, alternatively to direct analysis of fresh tissue collected during antidepressant treatment such as blood samples, some authors have developed interesting ex-vivo strategies using cultured cells that could mimic antidepressant response. For example, Gassen et al. demonstrated that peripheral mononuclear cells (PBMC) collected at the time of admission and treated with paroxetine demonstrated the same biological response (i.e. variation in DNMT1 phosphorylation) to antidepressant than PBMC collected before and after 6-week pharmacological treatment in patients (Gassen et al., 2015). Interestingly this approach allowed the use of mediator biomarkers in a predictive algorithm, as long term changes observed in patients after several weeks of treatment could be mimicked by a short term cell culture procedure.

To date, no commonly accepted biomarker, nor any combination of them, is available or about to become available for use in clinical practice in psychiatry, with the possible exception of kits that assess metabolizer status by genotyping cytochrome gene variants. Several explanations can be proposed. First, our knowledge of the genome is still progressing and new discoveries, new techniques and new bio-informatic challenges increase quickly both the possibility and the complexity of the field. Second, the development of biomarkers requires specific protocols to collect and store samples without bias, and to collect all relevant clinical data. Studies will need to be specifically designed for biomarker development, to be prospective and to include sufficiently large samples to avoid false discoveries. To this date, available studies have explored relatively small samples, and few included at least one replication cohort. The large majority of studies described average differences between groups, and did not explore the accuracy of proposed biomarkers in predicting or monitoring antidepressant response. However, this is a crucial characteristic of a future biomarker and an initial step before testing its utility in clinical practice and its potential to improve care and health costs. Finally, for the majority of candidate biomarkers, biological plausibility needs to be better explored. In the same vain, one could also hope that future studies will include not only one type of biomarker but an integrative approach including different biological levels, from epigenomic regulation of DNA to mRNA degradation. Although many studies have moved towards whole-genome or whole-transcriptome analyses, hypothesis-driven studies focusing on well-known candidate molecular markers or pathways remain interesting. Alterations at different levels of regulation appear to be emerging for some of

these candidates (i.e. gene expression level, DNA methylation and/or histone modification), encouraging integrative studies. However, these approaches would need to be complemented by studies without a priori hypotheses that allow for gene discovery.

Moreover, an important unexplored field of development in biomarker research is the “reverse” application of a validated biomarker, to better define phenotypic traits. This could also provide new avenues towards understanding the pathophysiology of depression and mechanisms of action of antidepressants.

While, to this date, biomarker development is still an ongoing work without clinical application, research networks and ambitious research programs (Lam et al., 2016; Trivedi et al., 2016) will probably in the future allow to move from a good idea to a new perspective for patients and health systems.

References:

Abe, N., Uchida, S., Otsuki, K., Hobara, T., Yamagata, H., Higuchi, F., Shibata, T., Watanabe, Y., 2011. Altered sirtuin deacetylase gene expression in patients with a mood disorder. Journal of Psychiatric Research 45, 1106-1112.

Allfrey, V.G., Faulkner, R., Mirsky, A.E., 1964. ACETYLATION AND METHYLATION OF HISTONES AND THEIR POSSIBLE ROLE IN THE REGULATION OF RNA SYNTHESIS. Proceedings of the National Academy of Sciences of the United States of America 51, 786-794.

Anacker, C., Zunszain, P.A., Carvalho, L.A., Pariante, C.M., 2011. The glucocorticoid receptor: Pivot of depression and of antidepressant treatment? Psychoneuroendocrinology 36, 415-425.

Bannister, A.J., Kouzarides, T., 2011. Regulation of chromatin by histone modifications. Cell Research 21, 381-395.

Baylin, S.B., Jones, P.A., 2011. A decade of exploring the cancer epigenome — biological and translational implications. Nature Reviews. Cancer 11, 726-734.

Belzeaux, R., Azorin, J.M., Ibrahim, E.C., 2014a. Monitoring candidate gene expression variations before, during and after a first major depressive episode in a 51-year-old man. BMC Psychiatry 14, 73.

Belzeaux, R., Bergon, A., Jeanjean, V., Loriod, B., Formisano-Treziny, C., Verrier, L., Loundou, A., Baumstarck-Barrau, K., Boyer, L., Gall, V., Gabert, J., Nguyen, C., Azorin, J.M., Naudin, J., Ibrahim, E.C., 2012. Responder and nonresponder patients exhibit different peripheral transcriptional signatures during major depressive episode. Transl Psychiatry 2, e185.

Belzeaux, R., Formisano-Treziny, C., Loundou, A., Boyer, L., Gabert, J., Samuelian, J.C., Feron, F., Naudin, J., Ibrahim, E.C., 2010. Clinical variations modulate patterns of gene expression and define blood biomarkers in major depression. J Psychiatr Res 44, 1205-1213.

Belzeaux, R., Lefebvre, M.N., Lazzari, A., Le Carpentier, T., Consoloni, J.L., Zendjidjian, X., Abbar, M., Courtet, P., Naudin, J., Boucraut, J., Gressens, P., Glaichenhaus, N., Ibrahim, E.C., 2017a. How to: Measuring blood cytokines in biological psychiatry using commercially available multiplex immunoassays. Psychoneuroendocrinology 75, 72-82.

Belzeaux, R., Lin, C.W., Ding, Y., Bergon, A., Ibrahim, E.C., Turecki, G., Tseng, G., Sibille, E., 2016. Predisposition to treatment response in major depressive episode: A peripheral blood gene coexpression network analysis. J Psychiatr Res 81, 119-126.

Belzeaux, R., Lin, R., Turecki, G., 2017b. Potential Use of MicroRNA for Monitoring Therapeutic Response to Antidepressants. CNS Drugs 31, 253-262.
Belzeaux, R., Loundou, A., Azorin, J.M., Naudin, J., Ibrahim, E.C., 2014b. Longitudinal monitoring of the serotonin transporter gene expression to assess major depressive episode evolution. Neuropsychobiology 70, 220-227.
Brinkman, A.B., Simmer, F., Ma, K., Kaan, A., Zhu, J., Stunnenberg, H.G., 2010. Whole-genome DNA methylation profiling using MethylCap-seq. Methods 52.
Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y., Allis, C.D., 1996. Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell 84, 843-851.
Cattaneo, A., Ferrari, C., Uher, R., Bocchio-Chiavetto, L., Riva, M.A., Consortium, M.R.C.I., Pariante, C.M., 2016. Absolute Measurements of Macrophage Migration Inhibitory Factor and Interleukin-1-beta mRNA Levels Accurately Predict Treatment Response in Depressed Patients. Int J Neuropsychopharmacol 19.
Cattaneo, A., Gennarelli, M., Uher, R., Breen, G., Farmer, A., Aitchison, K.J., Craig, I.W., Anacker, C., Zunsztain, P.A., McGuffin, P., Pariante, C.M., 2013. Candidate genes expression profile associated with antidepressants response in the GENDEP study: differentiating between baseline 'predictors' and longitudinal 'targets'. Neuropsychopharmacology 38, 377-385.
Chen, E.S., Ernst, C., Turecki, G., 2011. The epigenetic effects of antidepressant treatment on human prefrontal cortex BDNF expression. International Journal of Neuropsychopharmacology 14, 427-429.
Curradi, M., Izzo, A., Badaracco, G., Landsberger, N., 2002. Molecular Mechanisms of Gene Silencing Mediated by DNA Methylation. Molecular and Cellular Biology 22, 3157-3173.
deVos, T., Tetzner, R., Model, F., Weiss, G., Schuster, M., Distler, J., Steiger, K.V., Grützmann, R., Pilarsky, C., Habermann, J.K., Fleshner, P.R., Oubre, B.M., Day, R., Sledziewski, A.Z., Lofton-Day, C., 2009. Circulating Methylated SEPT9 DNA in Plasma Is a Biomarker for Colorectal Cancer. Clinical Chemistry 55, 1337.
Domschke, K., Tidow, N., Schwarte, K., Deckert, J., Lesch, K.-P., Arolt, V., Zwanzger, P., Baune, B.T., 2014. Serotonin transporter gene hypomethylation predicts impaired antidepressant treatment response. International Journal of Neuropsychopharmacology 17, 1167-1176.

Eckhardt, F., Lewin, J., Cortese, R., Rakyan, V.K., Attwood, J., Burger, M., Burton, J., Cox, T.V., Davies, R., Down, T.A., Haefliger, C., Horton, R., Howe, K., Jackson, D.K., Kunde, J., Koenig, C., Liddle, J., Niblett, D., Otto, T., Pettett, R., Seemann, S., Thompson, C., West, T., Rogers, J., Olek, A., Berlin, K., Beck, S., 2006. DNA methylation profiling of human chromosomes 6, 20 and 22. Nat Genet 38, 1378-1385.

El-Hage, W., Leman, S., Camus, V., Belzung, C., 2013. Mechanisms of antidepressant resistance. Front Pharmacol 4, 146.

Elliott, E., Ezra-Nevo, G., Regev, L., Neufeld-Cohen, A., Chen, A., 2010. Resilience to social stress coincides with functional DNA methylation of the Crf gene in adult mice. Nat Neurosci 13, 1351-1353.

Eyre, H.A., Lavretsky, H., Kartika, J., Qassim, A., Baune, B.T., 2016. Modulatory Effects of Antidepressant Classes on the Innate and Adaptive Immune System in Depression. Pharmacopsychiatry 49, 85-96.

Fabbri, C., Di Girolamo, G., Serretti, A., 2013. Pharmacogenetics of antidepressant drugs: an update after almost 20 years of research. Am J Med Genet B Neuropsychiatr Genet 162B, 487-520.

Falaleeva, M., Surface, J., Shen, M., de la Grange, P., Stamm, S., 2015. SNORD116 and SNORD115 change expression of multiple genes and modify each other's activity. Gene 572, 266-273.

Fiori, L.M., Turecki, G., 2016. Investigating epigenetic consequences of early-life adversity: some methodological considerations. Eur J Psychotraumatol 7, 31593.

Foster, J.A., McVey Neufeld, K.A., 2013. Gut-brain axis: how the microbiome influences anxiety and depression. Trends Neurosci 36, 305-312.

Freedman, J.E., Gerstein, M., Mick, E., Rozowsky, J., Levy, D., Kitchen, R., Das, S., Shah, R., Danielson, K., Beaulieu, L., Navarro, F.C., Wang, Y., Galeev, T.R., Holman, A., Kwong, R.Y., Murthy, V., Tanriverdi, S.E., Koupenova-Zamor, M., Mikhalev, E., Tanriverdi, K., 2016. Diverse human extracellular RNAs are widely detected in human plasma. Nat Commun 7, 11106.

Gadad, B.S., Jha, M.K., Czysz, A., Furman, J.L., Mayes, T.L., Emslie, M.P., Trivedi, M.H., 2017. Peripheral biomarkers of major depression and antidepressant treatment response: Current knowledge and future outlooks. J Affect Disord.

Gaiteri, C., Ding, Y., French, B., Tseng, G.C., Sibille, E., 2014. Beyond modules and hubs: the potential of gene coexpression networks for investigating molecular mechanisms of complex brain disorders. Genes Brain Behav 13, 13-24.
Gassen, N.C., Fries, G.R., Zannas, A.S., Hartmann, J., Zschocke, J., Hafner, K., Carrillo-Roa, T., Steinbacher, J., Preissinger, S.N., Hoeijmakers, L., Knop, M., Weber, F., Kloiber, S., Lucae, S., Chrousos, G.P., Carell, T., Ising, M., Binder, E.B., Schmidt, M.V., Ruegg, J., Rein, T., 2015. Chaperoning epigenetics: FKBP51 decreases the activity of DNMT1 and mediates epigenetic effects of the antidepressant paroxetine. Sci Signal 8, ra119.
Gladkevich, A., Kauffman, H.F., Korf, J., 2004. Lymphocytes as a neural probe: potential for studying psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry 28, 559-576.
Gross, J.A., Pacis, A., Chen, G.G., Barreiro, L.B., Ernst, C., Turecki, G., 2015. Characterizing 5-hydroxymethylcytosine in human prefrontal cortex at single base resolution. BMC Genomics 16, 672.
Group, F.-N.B.W., 2016. BEST (Biomarkers, EndpointS, and other Tools) Resource, in: (US), S.S.M.F.a.D.A. (Ed.).
Guilloux, J.P., Bassi, S., Ding, Y., Walsh, C., Turecki, G., Tseng, G., Cyranowski, J.M., Sibille, E., 2015. Testing the predictive value of peripheral gene expression for nonremission following citalopram treatment for major depression. Neuropsychopharmacology 40, 701-710.
Ha, M., Kim, V.N., 2014. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 15, 509-524.
Hennings, J.M., Uhr, M., Klengel, T., Weber, P., Putz, B., Touma, C., Czamara, D., Ising, M., Holsboer, F., Lucae, S., 2015. RNA expression profiling in depressed patients suggests retinoid-related orphan receptor alpha as a biomarker for antidepressant response. Transl Psychiatry 5, e538.
Himmerich, H., Milenovic, S., Fulda, S., Plumakers, B., Sheldrick, A.J., Michel, T.M., Kircher, T., Rink, L., 2010. Regulatory T cells increased while IL-1beta decreased during antidepressant therapy. J Psychiatr Res 44, 1052-1057.
Hobara, T., Uchida, S., Otsuki, K., Matsubara, T., Funato, H., Matsuo, K., Suetsugi, M., Watanabe, Y., 2010. Altered gene expression of histone deacetylases in mood disorder patients. Journal of Psychiatric Research 44, 263-270.

Hodgson, K., Tansey, K.E., Powell, T.R., Coppola, G., Uher, R., Zvezdana Dernovsek, M., Mors, O., Hauser, J., Souery, D., Maier, W., Henigsberg, N., Rietschel, M., Placentino, A., Aitchison, K.J., Craig, I.W., Farmer, A.E., Breen, G., McGuffin, P., Dobson, R., 2016. Transcriptomics and the mechanisms of antidepressant efficacy. Eur Neuropsychopharmacol 26, 105-112.

Iga, J., Ueno, S., Yamauchi, K., Motoki, I., Tayoshi, S., Ohta, K., Song, H., Morita, K., Rokutan, K., Ohmori, T., 2005. Serotonin transporter mRNA expression in peripheral leukocytes of patients with major depression before and after treatment with paroxetine. Neurosci Lett 389, 12-16.

Iga, J.-i., Ueno, S.-i., Yamauchi, K., Numata, S., Kinouchi, S., Tayoshi-Shibuya, S., Song, H., Ohmori, T., 2007. Altered HDAC5 and CREB mRNA expressions in the peripheral leukocytes of major depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry 31, 628-632.

Issler, O., Chen, A., 2015. Determining the role of microRNAs in psychiatric disorders. Nat Rev Neurosci 16, 201-212.

Iwasaki, Y.W., Siomi, M.C., Siomi, H., 2015. PIWI-Interacting RNA: Its Biogenesis and Functions. Annu Rev Biochem 84, 405-433.

Jenuwein, T., Allis, C.D., 2001. Translating the histone code. Science (New York, N.Y.) 293, 1074-1080.

Kang, H.-J., Kim, J.-M., Stewart, R., Kim, S.-Y., Bae, K.-Y., Kim, S.-W., Shin, I.-S., Shin, M.-G., Yoon, J.-S., 2013. Association of SLC6A4 methylation with early adversity, characteristics and outcomes in depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry 44, 23-28.

Kennedy, S.H., Lam, R.W., McIntyre, R.S., Tourjman, S.V., Bhat, V., Blier, P., Hasnain, M., Jollant, F., Levitt, A.J., MacQueen, G.M., McInerney, S.J., McIntosh, D., Milev, R.V., Muller, D.J., Parikh, S.V., Pearson, N.L., Ravindran, A.V., Uher, R., Group, C.D.W., 2016. Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 Clinical Guidelines for the Management of Adults with Major Depressive Disorder: Section 3. Pharmacological Treatments. Can J Psychiatry.

Kishore, S., Stamm, S., 2006. The snoRNA HBII-52 regulates alternative splicing of the serotonin receptor 2C. Science 311, 230-232.

Kohli, R.M., Zhang, Y., 2013. TET enzymes, TDG and the dynamics of DNA demethylation. Nature 502, 472-479.
Kornberg, R.D., Lorch, Y., 1999. Twenty-Five Years of the Nucleosome, Fundamental Particle of the Eukaryote Chromosome. Cell 98, 285-294.
Laird, P.W., 2003. The power and the promise of DNA methylation markers. Nat Rev Cancer 3, 253-266.
Lam, R.W., Milev, R., Rotzinger, S., Andreazza, A.C., Blier, P., Brenner, C., Daskalakis, Z.J., Dharsee, M., Downar, J., Evans, K.R., Farzan, F., Foster, J.A., Frey, B.N., Geraci, J., Giacobbe, P., Feilotter, H.E., Hall, G.B., Harkness, K.L., Hassel, S., Ismail, Z., Leri, F., Liotti, M., MacQueen, G.M., McAndrews, M.P., Minuzzi, L., Müller, D.J., Parikh, S.V., Placenza, F.M., Quilty, L.C., Ravindran, A.V., Salomons, T.V., Soares, C.N., Strother, S.C., Turecki, G., Vaccarino, A.L., Vila-Rodriguez, F., Kennedy, S.H., on behalf of the, C.A.N.B.I.T., 2016. Discovering biomarkers for antidepressant response: protocol from the Canadian biomarker integration network in depression (CAN-BIND) and clinical characteristics of the first patient cohort. BMC Psychiatry 16, 105.
Langfelder, P., Horvath, S., 2008. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9, 559.
Langie, S.A., Moisse, M., Declerck, K., Koppen, G., Godderis, L., Vanden Berghe, W., Drury, S., De Boever, P., 2016. Salivary DNA Methylation Profiling: Aspects to Consider for Biomarker Identification. Basic Clin Pharmacol Toxicol.
Lee, E.J., Banerjee, S., Zhou, H., Jammalamadaka, A., Arcila, M., Manjunath, B.S., Kosik, K.S., 2011. Identification of piRNAs in the central nervous system. RNA 17, 1090-1099.
Leuchter, A.F., Cook, I.A., Hamilton, S.P., Narr, K.L., Toga, A., Hunter, A.M., Faull, K., Whitelegge, J., Andrews, A.M., Loo, J., Way, B., Nelson, S.F., Horvath, S., Lebowitz, B.D., 2010. Biomarkers to predict antidepressant response. Curr Psychiatry Rep 12, 553-562.
Levenson, V.V., 2010. DNA methylation as a universal biomarker. Expert review of molecular diagnostics 10, 481-488.
Li, E., 2002. Chromatin modification and epigenetic reprogramming in mammalian development. Nat Rev Genet 3, 662-673.
Li, J.Z., Bunney, B.G., Meng, F., Hagenauer, M.H., Walsh, D.M., Vawter, M.P., Evans, S.J., Choudary, P.V., Cartagena, P., Barchas, J.D., Schatzberg, A.F., Jones, E.G., Myers, R.M.,

Watson, S.J., Jr., Akil, H., Bunney, W.E., 2013. Circadian patterns of gene expression in the human brain and disruption in major depressive disorder. Proc Natl Acad Sci U S A 110, 9950-9955.
Li, L., Choi, J.-Y., Lee, K.-M., Sung, H., Park, S.K., Oze, I., Pan, K.-F., You, W.-C., Chen, Y.-X., Fang, J.-Y., Matsuo, K., Kim, W.H., Yuasa, Y., Kang, D., 2012. DNA Methylation in Peripheral Blood: A Potential Biomarker for Cancer Molecular Epidemiology. Journal of Epidemiology 22, 384-394.
Lima, L., Mata, S., Urbina, M., 2005. Allelic isoforms and decrease in serotonin transporter mRNA in lymphocytes of patients with major depression. Neuroimmunomodulation 12, 299-306.
Lopez, J.P., Fiori, L.M., Cruceanu, C., Lin, R., Labonte, B., Cates, H.M., Heller, E.A., Vialou, V., Ku, S.M., Gerald, C., Han, M.H., Foster, J., Frey, B.N., Soares, C.N., Muller, D.J., Farzan, F., Leri, F., MacQueen, G.M., Feilotter, H., Tyryshkin, K., Evans, K.R., Giacobbe, P., Blier, P., Lam, R.W., Milev, R., Parikh, S.V., Rotzinger, S., Strother, S.C., Lewis, C.M., Aitchison, K.J., Wittenberg, G.M., Mechawar, N., Nestler, E.J., Uher, R., Kennedy, S.H., Turecki, G., 2017. MicroRNAs 146a/b-5 and 425-3p and 24-3p are markers of antidepressant response and regulate MAPK/Wnt-system genes. Nat Commun 8, 15497.
Lopez, J.P., Mamdani, F., Labonte, B., Beaulieu, M.-M., Yang, J.P., Berlim, M.T., Ernst, C., Turecki, G., 2013. Epigenetic regulation of BDNF expression according to antidepressant response. Molecular Psychiatry 18, 398-399.
Lui, L., Lowe, T., 2013. Small nucleolar RNAs and RNA-guided post-transcriptional modification. Essays Biochem 54, 53-77.
Lutz, P.-E., Turecki, G., 2014. DNA methylation and childhood maltreatment: from animal models to human studies. Neuroscience 264, 142-156.
Mamdani, F., Berlim, M.T., Beaulieu, M.M., Labbe, A., Merette, C., Turecki, G., 2011. Gene expression biomarkers of response to citalopram treatment in major depressive disorder. Transl Psychiatry 1, e13.
Mamdani, F., Berlim, M.T., Beaulieu, M.M., Turecki, G., 2014. Pharmacogenomic predictors of citalopram treatment outcome in major depressive disorder. World J Biol Psychiatry 15, 135-144.

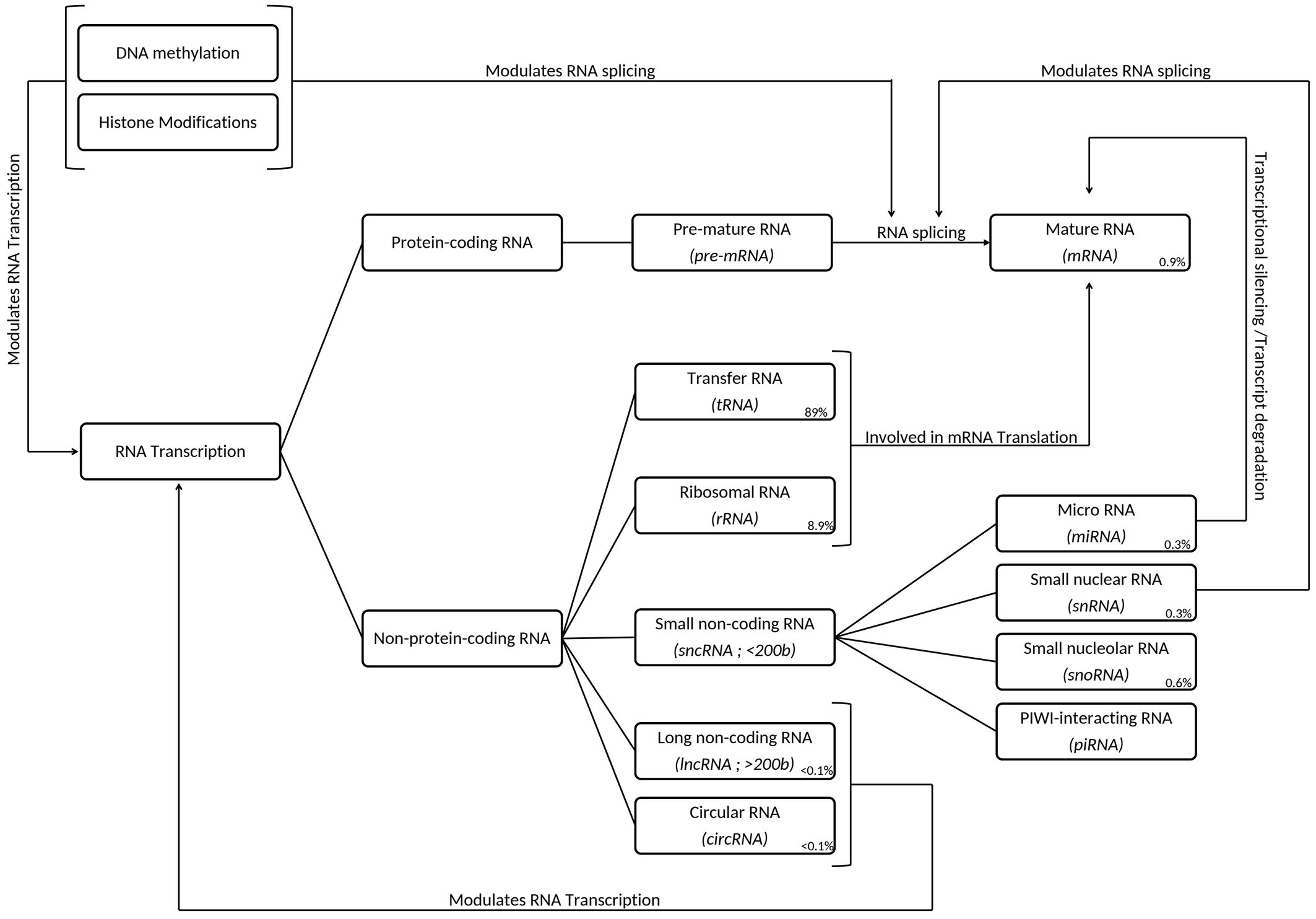
Mamrut, S., Harony, H., Sood, R., Shahar-Gold, H., Gainer, H., Shi, Y.-J., Barki-Harrington, L., Wagner, S., 2013. DNA Methylation of Specific CpG Sites in the Promoter Region Regulates the Transcription of the Mouse Oxytocin Receptor. PLOS ONE 8, e56869.
Maunakea, A.K., Chepelev, I., Zhao, K., 2010a. Epigenome mapping in normal and disease States. Circ Res 107, 327-339.
Maunakea, A.K., Nagarajan, R.P., Bilenky, M., Ballinger, T.J., D/'Souza, C., Fouse, S.D., Johnson, B.E., Hong, C., Nielsen, C., Zhao, Y., Turecki, G., Delaney, A., Varhol, R., Thiessen, N., Shchors, K., Heine, V.M., Rowitch, D.H., Xing, X., Fiore, C., Schillebeeckx, M., Jones, S.J.M., Haussler, D., Marra, M.A., Hirst, M., Wang, T., Costello, J.F., 2010b. Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature 466, 253-257.
Nanni, V., Uher, R., Danese, A., 2012. Childhood Maltreatment Predicts Unfavorable Course of Illness and Treatment Outcome in Depression: A Meta-Analysis. American Journal of Psychiatry 169, 141-151.
Nemeroff, C.B., 2016. Paradise Lost: The Neurobiological and Clinical Consequences of Child Abuse and Neglect. Neuron 89, 892-909.
Pérez, A., Castellazzi, Chiara L., Battistini, F., Collinet, K., Flores, O., Deniz, O., Ruiz, Maria L., Torrents, D., Eritja, R., Soler-López, M., Orozco, M., 2012. Impact of Methylation on the Physical Properties of DNA. Biophysical Journal 102, 2140-2148.
Pettai, K., Milani, L., Tammiste, A., Vosa, U., Kolde, R., Eller, T., Nutt, D., Metspalu, A., Maron, E., 2016. Whole-genome expression analysis reveals genes associated with treatment response to escitalopram in major depression. Eur Neuropsychopharmacol 26, 1475-1483.
Philibert, R., Glatt, S.J., 2017. Optimizing the chances of success in the search for epigenetic biomarkers: Embracing genetic variation. Am J Med Genet B Neuropsychiatr Genet.
Powell, T.R., Schalkwyk, L.C., Heffernan, A.L., Breen, G., Lawrence, T., Price, T., Farmer, A.E., Aitchison, K.J., Craig, I.W., Danese, A., Lewis, C., McGuffin, P., Uher, R., Tansey, K.E., D'Souza, U.M., 2013a. Tumor necrosis factor and its targets in the inflammatory cytokine pathway are identified as putative transcriptomic biomarkers for escitalopram response. Eur Neuropsychopharmacol 23, 1105-1114.
Powell, T.R., Smith, R.G., Hackinger, S., Schalkwyk, L.C., Uher, R., McGuffin, P., Mill, J., Tansey, K.E., 2013b. DNA methylation in interleukin-11 predicts clinical response to antidepressants in GENDEP. Transl Psychiatry 3, e300.

Quek, C., Bellingham, S.A., Jung, C.H., Scicluna, B.J., Shambrook, M.C., Sharples, R.A., Cheng, L., Hill, A.F., 2017. Defining the purity of exosomes required for diagnostic profiling of small RNA suitable for biomarker discovery. RNA Biol 14, 245-258.
Raison, C.L., Capuron, L., Miller, A.H., 2006. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol 27, 24-31.
Rajasethupathy, P., Antonov, I., Sheridan, R., Frey, S., Sander, C., Tuschl, T., Kandel, E.R., 2012. A role for neuronal piRNAs in the epigenetic control of memory-related synaptic plasticity. Cell 149, 693-707.
Robertson, K.D., Wolffe, A.P., 2000. DNA methylation in health and disease. Nat Rev Genet 1, 11-19.
Rothbart, S.B., Strahl, B.D., 2014. Interpreting thelanguage of histone and DNA modifications. Biochimica et biophysica acta 1839, 627-643.
Rush, A.J., Trivedi, M.H., Wisniewski, S.R., Nierenberg, A.A., Stewart, J.W., Warden, D., Niederehe, G., Thase, M.E., Lavori, P.W., Lebowitz, B.D., McGrath, P.J., Rosenbaum, J.F., Sackeim, H.A., Kupfer, D.J., Luther, J., Fava, M., 2006. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. Am J Psychiatry 163, 1905-1917.
Sullivan, P.F., Fan, C., Perou, C.M., 2006. Evaluating the comparability of gene expression in blood and brain. Am J Med Genet B Neuropsychiatr Genet 141B, 261-268.
Tadic, A., Muller-Engling, L., Schlicht, K.F., Kotsiari, A., Dreimuller, N., Kleimann, A., Bleich, S., Lieb, K., Frieling, H., 2014. Methylation of the promoter of brain-derived neurotrophic factor exon IV and antidepressant response in major depression. Mol Psychiatry 19, 281-283.
Taunton, J., Hassig, C.A., Schreiber, S.L., 1996. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science (New York, N.Y.) 272, 408-411.
The, B.c., 2016. Quantitative comparison of DNA methylation assays for biomarker development and clinical applications. Nat Biotech 34, 726-737.
Trivedi, M.H., McGrath, P.J., Fava, M., Parsey, R.V., Kurian, B.T., Phillips, M.L., Oquendo, M.A., Bruder, G., Pizzagalli, D., Toups, M., Cooper, C., Adams, P., Weyandt, S., Morris, D.W., Grannemann, B.D., Ogden, R.T., Buckner, R., McInnis, M., Kraemer, H.C., Petkova, E., Carmody, T.J., Weissman, M.M., 2016. Establishing moderators and biosignatures of

antidepressant response in clinical care (EMBARC): Rationale and design. J Psychiatr Res 78, 11-23.
Tsankova, N.M., Berton, O., Renthal, W., Kumar, A., Neve, R.L., Nestler, E.J., 2006. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nature Neuroscience 9, 519-525.
Tsao, C.W., Lin, Y.S., Chen, C.C., Bai, C.H., Wu, S.R., 2006. Cytokines and serotonin transporter in patients with major depression. Prog Neuropsychopharmacol Biol Psychiatry 30, 899-905.
Turecki, G., 2014. The molecular bases of the suicidal brain. Nat Rev Neurosci 15, 802-816.
Valadi, H., Ekstrom, K., Bossios, A., Sjostrand, M., Lee, J.J., Lotvall, J.O., 2007. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 9, 654-659.
Verbelen, M., Weale, M.E., Lewis, C.M., 2017. Cost-effectiveness of pharmacogenetic-guided treatment: are we there yet? Pharmacogenomics J.
Vinson, C., Chatterjee, R., 2012. CG methylation. Epigenomics 4, 655-663.
Wahid, F., Shehzad, A., Khan, T., Kim, Y.Y., 2010. MicroRNAs: synthesis, mechanism, function, and recent clinical trials. Biochim Biophys Acta 1803, 1231-1243.
Weber, M., Hellmann, I., Stadler, M.B., Ramos, L., Paabo, S., Rebhan, M., Schubeler, D., 2007. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat Genet 39, 457-466.

Figure legend:

FIGURE 1: Classification and relation between different gene expression and epigenomic biomarkers.
% corresponds to typical relative abundance of different RNA species in mammalian tissues


DNA methylation
Histone Modifications
Modulates RNA splicing
Modulates RNA splicing
Modulates RNA Transcription
Transcriptional silencing /Transcript degradation
Protein-coding RNA
Pre-mature RNA
(pre-mRNA)
RNA splicing
Mature RNA
(mRNA)
0.9%
RNA Transcription
Transfer RNA
(tRNA)
89%
Involved in mRNA Translation
Ribosomal RNA
(rRNA)
8.9%
Micro RNA
(miRNA)
0.3%
Small nuclear RNA
(snRNA)
0.3%
Non-protein-coding RNA
Small non-coding RNA
(sncRNA ; <200b)
Small nucleolar RNA
(snoRNA)
0.6%
PIWI-interacting RNA
(piRNA)
Long non-coding RNA
(lncRNA ; >200b)
<0.1%
Circular RNA
(circRNA)
<0.1%
Modulates RNA Transcription

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

Contributors

All authors designed and conducted the literature search and wrote the manuscript.

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