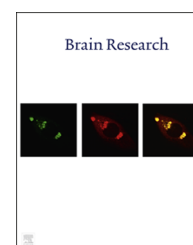


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Review

Brain derived neurotrophic factor: Epigenetic regulation in psychiatric disorders

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ABSTRACT

Brain Derived Neurotrophic Factor (BDNF) is a neurotrophin with important functions in neuronal development and neuroplasticity. Accumulating evidence suggests that alterations in BDNF expression levels underlie a variety of psychiatric and neurological disorders. Indeed, BDNF therapies are currently being investigated in animal models and clinical studies. However, very little is currently known about the mechanisms that deregulate BDNF gene expression in these disorders. The BDNF gene structure and tissue expression pattern is complex, controlled in humans by 9 different gene promoters. Recently, epigenetic changes at the BDNF gene locus have been proposed to provide a link between gene and environment. In this review, we will summarize the current knowledge of BDNF epigenetic regulation with respect to psychiatric disorders and describe how this information can be applied in therapy and future research.

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1. Introduction

The neurotrophin family consists of 4 members with important functions during nervous system development and neuronal plasticity. Of these, BDNF has the most abundant and widespread expression in the mammalian brain (Murer et al., 1999; Pruunsild et al., 2007). BDNF is released from neurons both pre- and postsynaptically, either constitutively or in an activity-dependent manner (Lessmann and Brigadski, 2009). Secreted BDNF can interact with two receptors: the p75 neurotrophin receptor (p75NTR) and the tropomyosin-related kinase receptor B (TrkB). Signaling by BDNF depends on the proteolytical cleavage of a pro-form of BDNF to a mature form. Whereas proBDNF binds preferentially to p75NTR, mediating apoptosis and long-term depression, mature BDNF binds to TrkB and stimulates downstream signaling pathways leading to a plethora of effects: neuronal differentiation, outgrowth of neurites, increased cell survival and strengthening of synapses (Barker, 2009; Lu et al., 2005). Due to its role in neurogenesis and long term potentiation, BDNF signaling in the limbic structures and cerebral cortex is central for learning and memory (Cunha et al., 2010).

Disruption of BDNF signaling in the brain, mainly due to decreases in expression or release, has been linked in recent years to a range of psychiatric and neurological disorders (Balaratnasingam and Janca, 2012). A polymorphism in the coding region for the prodomain of BDNF, called Val66Met, is associated with memory impairment in humans (Baj et al., 2013; Egan et al., 2003; Hariri et al., 2003). In vitro studies suggest that the polymorphism leads to decreased BDNF release (Chen et al., 2004; Egan et al., 2003). BDNF replacement therapy is actively being pursued in human and animal models of diseases, including Huntington's disease, Alzheimer's disease and depression (Nagahara and Tuszynski, 2011). Furthermore, antidepressant treatment has been shown to increase levels of serum BDNF in depressed patients (Dwivedi, 2009).

Due to the central role of BDNF in brain development and plasticity, early environmental effects on BDNF levels may have long-term effects on brain activity. Indeed, it is well

known that childhood trauma can lead to psychiatric disorders in adults and that BDNF gene expression is reduced by acute and chronic stress, as covered by recent reviews (Balaratnasingam and Janca, 2012; Boulle et al., 2012). The mechanism leading to BDNF gene down-regulation is, however, currently unclear. Recent work in rodents suggests an epigenetic mechanism whereby environmental effects – such as fear conditioning, electroconvulsive seizure, early-life adversity and drug treatment – are associated with a change in *Bdnf* gene expression (Boulle et al., 2012; Roth and Sweatt, 2011). This review will focus on the human BDNF gene and highlight research results from recent studies of the epigenetic regulation of BDNF in human subjects.

2. Human BDNF gene regulation

2.1. Complex gene structure and expression

The human BDNF gene locus is complex, consisting of 11 exons and 9 different promoters (Pruunsild et al., 2007) (Table 1). Additional complexity is present due to alternative splice sites in exons II and IX, and two alternative polyadenylation sites in exon IX. The coding region for mature BDNF is in exon IX, which is present in all splice forms. Start codons for translation are present in exons I, VII, VIII and IX, leading to variations in the N-terminal signal peptide sequence of the corresponding pre-proBDNF forms. Furthermore, an anti-sense transcript is synthesized from the opposite DNA strand and may regulate BDNF transcript levels (Modarresi et al., 2012; Pruunsild et al., 2007).

Whereas all human BDNF alternative mRNA transcripts are highly expressed in the brain, some are also expressed in non-neuronal tissue (Pruunsild et al., 2007). For example, expression of exon I-containing transcripts is also high in the testis. Transcripts containing exons Vh, VI and IX have a widespread expression in peripheral tissues, whereas transcripts containing exons II, III, IV, V and VII are predominantly brain-specific (Table 1). Expression of BDNF transcripts in the human prefrontal cortex peaks in the first few years of life, consistent with

Table 1 – Characterization of the human BDNF gene locus.

Exon	Promoter ^a	CpG island ^b	ATG ^a	Tissue expression ^a	In vivo induction ^c	In vitro induction ^d
I	Yes	Yes	Yes	Brain and testis	Highest	Highest
II	Yes	Yes		Brain only		
III	Yes			Mainly brain-specific		
IV	Yes	CpG-rich		Mainly brain-specific	Highest	Highest
V	Yes			Mainly brain-specific		
Vh	Yes			Brain and few other tissues		
VI	Yes	Yes		Widespread		
VII	Yes		Yes	Brain only		
VIII			Yes			
VIIIh						
IX	Yes		Yes	Widespread	Highest	

Vh and VIIIh are specific to the human BDNF gene.

^a Data from Pruunsild et al. (2007).

^b Data from Boulle et al. (2012).

^c Data from Koppel et al. (2009).

^d Data from Pruunsild et al. (2011).

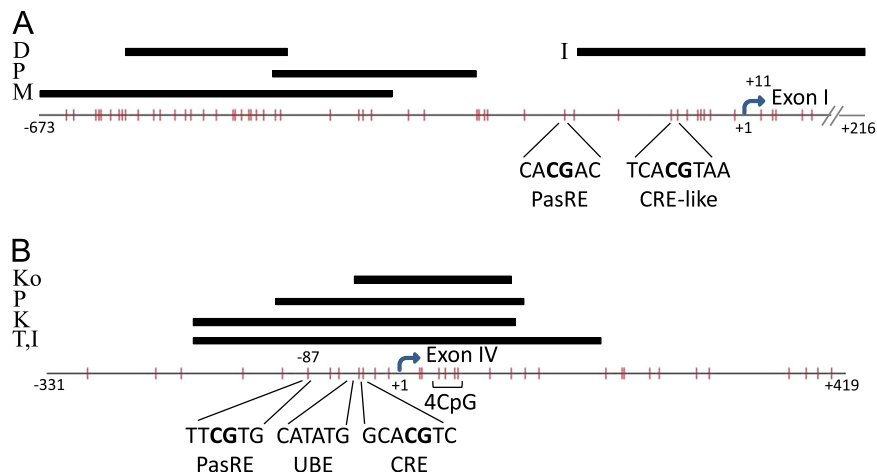


Fig. 1 – Genomic structure of the human BDNF promoters I (A) and IV (B). The positions of CpG dinucleotides are marked with vertical lines. The 5'-most transcriptional start sites (Pruunsild et al., 2007) are marked with an arrow. Cis-acting regulatory elements (Pruunsild et al., 2011) are indicated below the DNA strand. The positions of the PCR amplicons analyzed in the following studies are indicated above the DNA strand by the code: D=D'Addario et al., 2012 and 2013; P=Perroud et al., 2013; M=Mill et al., 2008; Ko=Kordi-Tamandani et al., 2012; K=Keller et al., 2010; T=Tadic et al., 2014; I=Ikemage et al., 2013b. The four CpG sites specifically studied in Keller et al., 2010 are indicated (4CpG).

a role in early development (Wong et al., 2009). Generation of alternative transcripts allows differential regional and subcellular mRNA targeting, as well as promoter-specific responses to environmental stimuli (Tongiorgi, 2008).

2.2. Transcriptional regulation

Most of the studies on BDNF gene regulation have been carried out in rodents, where current evidence suggests that neuronal activity acts via calcium signaling to stimulate transcription at *Bdnf* promoters I and IV (Greer and Greenberg, 2008; West et al., 2001). Regulation of the human BDNF gene in an in vivo context has been examined in bacterial artificial chromosome (BAC)-transgenic mice (Koppel et al., 2009). This study showed that a 168 kb region spanning the human BDNF locus contains all the promoter-regulatory elements necessary for tissue-specific reporter gene expression. In addition, the human BDNF transgene was inducible by kainic acid, similarly to the endogenous mouse *Bdnf* gene. Highest induction levels in both hippocampus and cortex were observed for human transcripts which had exons I, IV or IX as the 5'-exon (Table 1). This is the first indication that neural activity regulates human BDNF gene expression in vivo.

A recent study has examined the transcription factors and cis-elements required for activity-dependent transcription of the human BDNF gene (Pruunsild et al., 2011). First, it was demonstrated that KCl stimulation of primary cortical neurons, taken from BAC-transgenic mice with human BDNF inserted in the genome, led to upregulation of human BDNF transcripts. Next, rat primary cortical neurons were transfected with human promoter-reporter constructs and subjected to membrane depolarization by treatment with KCl. In both assays, human promoters I and IV were most upregulated by KCl-depolarization (Table 1). Mutation of a cAMP/Ca²⁺-response element (CRE)-like element in promoter I lowered the basal promoter activity but did not affect induction by KCl. Deletion and mutation analysis identified an E-box-like

bHLH-PAS transcription factor response element (PasRE) in the proximal promoter region that was sufficient for induction of the human BDNF promoter I by KCl-depolarization (Fig. 1A). Furthermore, it was demonstrated that this element bound a dimer of ARNT2 and NPAS4, two bHLH-PAS transcription factors, by mobility shift assay, antibody supershift and by co-transfection with expression plasmids for the native or dominant-negative ARNT2 and NPAS4 proteins. Based on their results, the authors suggest that the CRE-like element is important for basal activity, where NPAS4 levels are limiting. Neuronal depolarization leads to an increase in NPAS4 levels and thereby increased ARNT2-NPAS4 heterodimer formation, leading to increased expression from the exon I promoter. At the exon IV promoter, the most important cis-regulatory elements for KCl-mediated induction were determined to be a CRE, a USF-binding element (UBE) and a PasRE, which bind CRE-binding protein (CREB), USF and ARNT2-NPAS4, respectively (Fig. 1B). Whereas the CRE element was needed for the initial transcriptional induction by KCl-depolarization, the PasRE element was shown to be essential for further enhancement of human BDNF promoter IV induction. Importantly, chromatin immunoprecipitation studies showed direct binding of ARNT2 to endogenous human BDNF promoters I and IV, and CREB to promoter IV, in human postmortem parietal cortex samples. The importance of neuronal activity-induced NPAS4 expression in controlling BDNF mRNA expression from promoters I and IV is supported by rodent studies (Bloodgood et al., 2013; Lin et al., 2008; Ramamoorthi et al., 2011).

3. Epigenetic regulation of BDNF in psychiatric disorders

3.1. Epigenetics – a link between gene and environment

Discovery of the DNA double helix and the effect of irreversible changes in the DNA sequence, such as point mutations

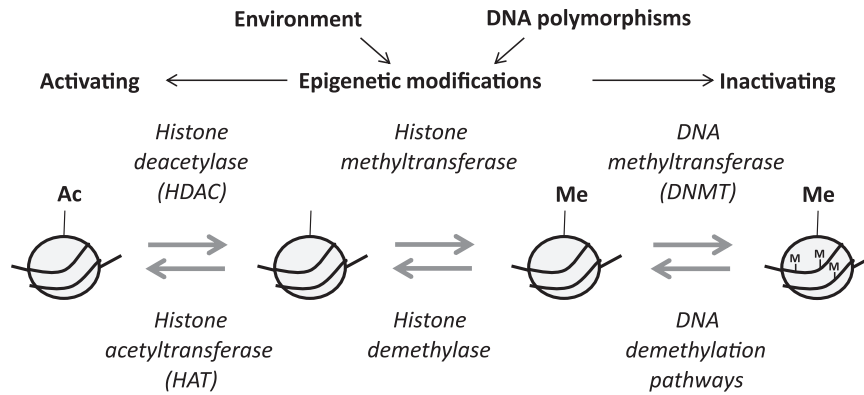


Fig. 2 – A simplified model for how reversible epigenetic modifications regulate transcriptional activity of genes. Acetylated histones (Ac) are associated with highly active genes, whereas methylation of histones (Me) at specific lysines (e.g. H3K9 or H3K27) is an inactivating signal. Methylation of cytosines at CpG dinucleotides in DNA (M) is also inactivating. Epigenetic modifications are subject to environmental and genetic effects.

and deletions, were groundbreaking. However, it soon became obvious that a second layer of gene regulation existed, as somatic cells display different gene expression profiles despite having the same genetic potential – this layer was termed “epigenetics”. The modern definition of epigenetics is “the study of mitotically and/or meiotically heritable changes in gene expression not caused by changes in the DNA sequence” (Feinberg and Tycko, 2004). In recognition of the widespread usage of the term to describe metastable or transient changes in chromatin, a recent definition is based on the structural adaptation of chromosomal regions, without the requirement for heritability (Bird, 2007).

DNA is wrapped around nucleosomes, which each consist of 8 histone protein cores that are highly basic. All the histone proteins can be modified and the modifications have two main mechanisms of action: influencing the overall structure of chromatin or regulating the binding of effector molecules (Bannister and Kouzarides, 2011). One of these modifications is histone acetylation, which influences chromatin structure. Histone acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs catalyze the transfer of an acetyl group from acetyl CoA to the ϵ -amino group of histone lysine side chains. This neutralizes the lysine's positive charge and weakens the interactions between the negative DNA backbone and the histone, making the DNA more accessible for the transcription machinery (Fig. 2). Acetylation is enriched at enhancer elements and gene promoters, where it is likely to facilitate access of transcription factors (Wang et al., 2008). HDAC enzymes remove lysine acetylation, thereby restoring the positive charge of the lysine and stabilizing local chromatin architecture. Lysine residues on histones can be methylated by histone methyltransferases (Fig. 2). The effect of histone methylation on gene transcription depends on the particular site of methylation. Methylation of histone H3 at lysine 9 (H3K9) and 27 (H3K27) are normally repressive, via the recruitment of chromatin-modifying activities such as DNA methyltransferases (DNMTs) (Cedar and Bergman, 2009). Note that methylation of histones at other sites (e.g. H3K4) may be activating, and that bivalent histone methylation marks exist, e.g. H3K4 and H3K27 trimethylation on poised gene

promoters in embryonic stem cells (Bernstein et al., 2006; Zhou et al., 2011).

DNA methylation correlates with gene repression and is perhaps the best studied epigenetic mechanism (Bergman and Cedar, 2013). DNA methylation involves addition of a methyl group to cytosine and occurs, in mammals, almost solely in association with CpG dinucleotides. CpG dinucleotides tend to cluster in CpG islands defined as regions of more than 200 bases that possess a C+G content of minimum 50% (Portela and Esteller, 2010). Methylated CpG islands are typically associated with silent DNA whereas unmethylated CpG islands are targets for transcription factors. About 40–50% of human genes have CpG islands within or close to their promoter, the majority of which are unmethylated in normal cells (Zhu and Yao, 2007).

DNA methylation is carried out by the DNMT family of enzymes that catalyze the transfer of a methyl group from S-adenosyl methionine to cytosine in DNA (Fig. 2). Mammals possess five DNMT family members: DNMT1, DNMT2, DNMT3A, DNMT3B and DNMT3L, but only DNMT1, DNMT3A and DNMT3B have methyltransferase activity (Portela and Esteller, 2010). The enzymes are categorized into de novo DNMTs (DNMT3A and DNMT3B) and maintenance DNMTs (DNMT1). DNMT3A and DNMT3B are considered de novo enzymes because they are believed to establish the pattern of methylation during embryonic development and are highly expressed in embryonic stem cells while downregulated in differentiated cells (Okano et al., 1999). DNMT1 has a preference for hemi-methylated DNA produced via semi-conservative replication but also has de novo activity (Hermann et al., 2004). The mechanism by which transcription is repressed after DNA methylation is established is not entirely clear, but proteins with a methyl-CpG binding domain are involved (El-Osta et al., 2001). One such protein, methyl-CpG-binding protein 2 (MECP2), binds methylated cytosine and recruits HDAC, leading to transcriptional repression (Nan et al., 1998). Methylation can also directly inhibit the access of transcription factors (Kumari and Usdin, 2001). Gene-specific demethylation is likely to be directed by HATs, which in turn are recruited by specific *trans*-acting factors (Bergman and Cedar, 2013). The TET family of

5-methylcytosine (5 mC) hydroxylases convert 5 mC to 5-hydroxymethylcytosine (5 hmC) as a key initiating step in active demethylation (Gavin et al., 2013; Guo et al., 2011; Tahiliani et al., 2009).

Most epigenetic modifications are believed to be erased with each generation; however, there have been reports of changes in DNA methylation that persist in offspring in mammals (Daxinger and Whitelaw, 2012). Immediately after fertilization, the paternal genome undergoes rapid DNA demethylation and histone modifications, whereas the maternal genome is gradually demethylated. Eventually, embryonic methylation is established (Morgan et al., 2005). On imprinted genes, the pattern of methylation is parent-specific, indicating that chromatin marks can survive embryonic reprogramming (Bergman and Cedar, 2013). Both environmental and genetic factors affect epigenetic modifications in the brain, providing a mechanism for gene-environment interactions in psychiatric disorders (Petronis, 2010; Rutten and Mill, 2009; Tycko, 2010). For example, DNA

polymorphisms may alter the ability of a specific locus to be epigenetically modified in response to an environmental insult (Rutten and Mill, 2009).

3.2. A role for epigenetic regulation of BDNF in psychiatric disorders?

In recent years, there has been an explosion of interest in studying BDNF epigenetic regulation in human subjects (see Table 2). This is partly related to the promising experimental results from rodent models and initial human postmortem studies, and partly due to the widespread acceptance that peripheral blood cells can be used as a proxy for brain tissue (Bouille et al., 2012; Davies et al., 2012). The first paper in humans, published in 2008, examined DNA methylation in postmortem brain tissue of major psychosis (schizophrenia and bipolar disorder) subjects and matched controls (Mill et al., 2008). There were no significant psychosis-associated methylation differences in exon IX or three other

Table 2 – Studies of epigenetic regulation of the BDNF gene in human subjects with relevance for psychiatric disorders.

Test group	Variable	Epigenetic changes	BDNF promoter	Tissue	Reference
Schizophrenia, bipolar disorder and control subjects	Val66Met SNP	Decrease in DNA methylation	IX (exonic)	Frontal cortex	Mill et al. (2008)
Schizophrenic patients vs. healthy controls	Schizophrenia	Decrease in DNA methylation	IV	Blood cells	Kordi-Tamandani et al. (2012)
Schizophrenic patients vs. healthy controls	Schizophrenia	Increase in DNA methylation at specific CpG	I	Blood cells	Ikegame et al. (2013b)
Suicidal vs. non-suicidal death	Suicidal death	Increase in DNA methylation at specific CpG sites	IV	Wernicke area	Keller et al. (2010)
Depressed patients vs. healthy controls	Depression	Increase/decrease in DNA methylation at specific CpG sites	I	Blood cells	Fuchikami et al. (2011)
Depressed patients vs. healthy controls	Depression	Increase in DNA methylation	I	Blood cells	D'Addario et al. (2013)
Depressed patients	Antidepressant treatment	Decrease in H3K27 trimethylation	IV	Frontal cortex	Chen et al. (2011)
Depressed patients	Antidepressant treatment response	Decrease in H3K27 trimethylation	IV	Blood cells	Lopez et al. (2013)
Depressed patients	Treatment with lithium or valproate	Decrease in DNA methylation	I	Blood cells	D'Addario et al. (2013)
Depressed patients	Antidepressant treatment response	Predicted by DNA methylation at specific CpG	IV	Blood cells	Tadic et al. (2014)
Depressed patients	Suicidal behavior	Increase in DNA methylation	VI (exonic)	Blood cells	Kang et al. (2013)
Bipolar disorder patients vs. healthy controls	Bipolar disorder	Increase in DNA methylation	NS	Frontal cortex	Rao et al. (2012)
Bipolar disorder II patients vs. healthy controls	Bipolar disorder II	Increase in DNA methylation	I	Blood cells	D'Addario et al. (2012)
Bipolar disorder I+II patients	Treatment with lithium or valproate	Decrease in DNA methylation	I	Blood cells	D'Addario et al. (2012)
BPD subjects vs. healthy controls	Borderline personality disorder	Increase in DNA methylation	I and IV	Blood cells	Perroud et al. (2013)
BPD subjects	Psychotherapy response	Decrease in DNA methylation	I and IV	Blood cells	Perroud et al. (2013)

BPD=borderline personality disorder and NS=not stated.

tested regions (upstream of exons I and VI and immediately downstream of exon III). However, the authors noted that the Val66Met SNP in exon IX, which removes a CpG site, is associated with a decrease in DNA methylation in adjacent CpG sites, when all tested samples were grouped by genotype. Two other papers have examined methylation status in the peripheral blood cells of schizophrenic patients, compared to healthy controls, with an observed decrease in DNA methylation in the exon IV promoter (Kordi-Tamandani et al., 2012) and a modest increase in methylation of a specific CpG in the exon I promoter (located at +11 in Fig. 1A) (Ikegame et al., 2013b). In the latter study, there was no difference in DNA methylation at the exon IV promoter.

A key study within this field examined *BDNF* promoter IV methylation in postmortem brain tissue of suicide completers (Keller et al., 2010). Importantly, the analyzed gene region was mainly unmethylated or low methylated in control and suicide tissue, which is consistent with it being a CpG island. Using 3 different analysis techniques, four specific CpG sites downstream of the transcription start site were identified with increased methylation in tissue from suicide victims as compared to control subjects (Fig. 1B). In 33 controls, the mean methylation at these 4 CpG sites was always less than 13.0%, whereas in 13 of the 44 suicide victims it was 13.1–34.2%. Furthermore, in a representative subgroup of samples, it was shown that *BDNF* mRNA levels were inversely correlated with the mean degree of methylation at these 4 CpG sites, suggesting that DNA methylation at these sites may be involved in transcriptional repression *in vivo*. Although 70% of the suicide victims had no psychiatric diagnosis, this study identified the region surrounding the *BDNF* exon IV transcription start site as potentially of interest in psychiatric disorders, such as major depression.

DNA methylation at the *BDNF* exon I and IV promoter regions were analyzed in peripheral blood cells of depressed vs. healthy subjects, using a MassARRAY technique (Fuchikami et al., 2011). Technical difficulties reduced the targeted CpG sites for methylation analysis from 81 to 35 informative CpG sites for exon I, and from 28 to 19 informative CpG sites for exon IV promoter regions, respectively. Nonetheless, a methylation profile was identified for the exon I promoter, but not for exon IV, in depressed patients that was distinct from healthy controls. Thus, this study points to DNA methylation profiling at the *BDNF* exon I promoter as a potential diagnostic biomarker for major depression. In another study, methylation of the exon I promoter in blood cells was shown to be increased in depressed patients, compared to healthy controls, and was decreased in patients receiving mood stabilizers in combination with antidepressants, as opposed to antidepressants alone (D'Addario et al., 2013). Also in blood cells, DNA methylation status at a specific CpG at baseline predicted the final response to antidepressant therapy (Tadic et al., 2014). Interestingly, the predictive CpG lies at –87 in the middle of the PasRE element (Fig. 1B). Another study in peripheral blood demonstrated that *BDNF* methylation status at a CpG island in exon VI was associated with suicidal behavior in depressed patients (Kang et al., 2013).

In bipolar disorder, hypermethylation of an unspecified *BDNF* promoter region was observed in postmortem brain

tissue, compared to healthy controls (Rao et al., 2012). Analysis of blood cells demonstrated higher levels of DNA methylation at the exon I promoter in bipolar disorder II, compared to either healthy controls or bipolar disorder I. In both bipolar disorder I and II patients, treatment with mood stabilizers was associated with lower methylation levels (D'Addario et al., 2012).

Both exon I and IV promoter regions were examined for DNA methylation in peripheral blood cells from borderline personality disorder (BPD) subjects and healthy controls. Higher levels of DNA methylation were observed in both regions in BPD, compared to healthy controls. In BPD subjects, a decrease in methylation status was observed in responders to a 4-week psychotherapy course, whereas non-responders exhibited increased methylation (Perroud et al., 2013).

A summary of the regions queried for DNA methylation at *BDNF* exons I and IV, in selected studies, is presented in Fig. 1. Three studies used methylation-specific PCR, which detects only DNA methylation at the binding sites of the forward and reverse primers (D'Addario et al., 2012, 2013; Kordi-Tamandani et al., 2012). One study tested only for methylation at the methyl-sensitive restriction sites used (Rao et al., 2012). The overall methylation level in the PCR-amplified regions was analyzed in another study (Perroud et al., 2013). The remaining studies identified DNA methylation at specific CpG sites. It is tempting to speculate that the DNA methylation changes observed lead to altered binding of general and sequence-specific transcription factors to promoters I and IV, although this awaits further research.

There are few studies examining the role of histone modifications at the *BDNF* gene promoter in humans. In postmortem brain tissue, the transcriptional up-regulation of *BDNF* which occurs from fetal to childhood and/or young adult stages was shown to be accompanied by increases in H3K4 trimethylation, a mark of active chromatin, at promoters I and IV (Mellios et al., 2008). In depressed patients, two studies suggest that antidepressants may regulate *BDNF* expression through alterations in the H3K27 methylation state at promoter IV: In postmortem brain tissue, H3K27 methylation was reduced in patients that had used antidepressants, compared to patients free of antidepressants (Chen et al., 2011). In blood cells, a prospective study in treatment-naïve depressive subjects revealed a decrease in H3K27 methylation in responders, but not in non-responders, after 8 weeks of antidepressant treatment (Lopez et al., 2013).

3.3. Research challenges and limitations

Epigenetics has become a popular topic in psychiatric research and has the potential to create a bridge in the nature vs. nurture discussion, but the research is not without challenges and limitations. One obvious limitation is the fact that it is not possible to investigate DNA methylation in the brain tissue of live subjects. For investigations stretching over time, researchers rely largely on peripheral blood. It is difficult to conclude to what extent DNA methylation from blood tissue reflects changes in the brain, as blood cells stem from mesoderm and brain cells originate from ectodermic tissue. If the environmental factor suspected of influencing

DNA methylation takes place during fetal development, such as maternal smoking during pregnancy, the use of DNA methylation in leukocytes as proxy for brain tissue is justified. However, if the environmental influence happens after birth, the changes in DNA methylation are more likely to be tissue-specific (Heijmans and Mill, 2012). Alternatively, the environmental influence may generate a soluble factor, e.g. a hormone, which can influence DNA methylation more broadly.

Similarity between DNA methylation in whole blood and regions of the brain was investigated (Davies et al., 2012). Postmortem brain samples from healthy subject were obtained together with whole blood samples, taken prior to death, from a subset of the subjects. The authors discovered distinct tissue-specific differences in DNA methylation, especially in genes involved in neuronal development and differentiation, including an intronic region in *BDNF*. The tissue-specific variation was predominant in intragenic CpG islands, CpG island shores, and in promoters with low CG density, whereas CpG islands in promoters were generally hypomethylated. Inter-individual differences in DNA methylation were greatest in blood and lowest in cortex. They did find a correlation between individual DNA methylation in blood and brain but as the authors mention, it cannot be excluded that the individual differences observed are a result of genomic variation. At the *BDNF* locus, the Val66Met polymorphism has been shown to alter DNA methylation, as Val/Val homozygotes have a higher, though modest, DNA methylation level around the site than individuals with Met (Mill et al., 2008). It was concluded by Davies et al. (2012) that even though differences between tissues exceed inter-individual differences within each tissue, peripheral blood may have some applicability for studies querying methylation patterns in the brain. In another study, comparison of the methylation of 8 specific loci in leukocytes and buccal cells, which are ectodermic of origin, showed similar results in half of the loci (Talens et al., 2010). This also provides justification for the use of peripheral tissue as proxy for brain DNA methylation, at least for some loci. One thing that can complicate the interpretation of DNA methylation in blood is that different types of leukocytes have slightly different DNA methylation profiles (Heijmans and Mill, 2012). Leukocyte distribution varies between persons and blood composition is affected markedly by infection, which can make a blood cell count necessary. It is then up to the researcher to decide how to correct for cell count differences or when to exclude subjects.

Another issue is that knowledge regarding DNA methylation stability is scarce. Flexibility of DNA methylation likely varies between individual sites and environmental factors might influence the ability to rapidly methylate or demethylate certain sites. This dynamic regulation is not captured in postmortem samples but blood samples can potentially clarify this phenomenon (Lutz and Turecki, 2014). Many confounders known to alter DNA methylation, e.g. smoking, diet, and stress, could influence the results of clinical epigenetic studies (Philibert et al., 2013; Unternaehrer et al., 2012; Widiker et al., 2010). In a study designed to measure dynamic changes in DNA methylation of the *BDNF* gene, blood samples from 76 healthy participants were taken before (pre-stress), 10 min (post-stress), and 90 min after (follow-up) a Trier

social stress test. The target sequence of *BDNF* was in the 3' region of exon VI, reported to lie within a CpG island that includes exons V, Vh and VI. Overall DNA methylation of the *BDNF* target sequence was found to be stable across the 3 time points, suggesting that psychosocial stress does not have an acute epigenetic effect on the investigated *BDNF* region (Unternaehrer et al., 2012).

In the interpretation of DNA methylation data, it would be helpful with knowledge about the normal variation of methylation levels for that particular locus. Variation, pattern and stability of DNA methylation was investigated for 16 candidate loci, selected because of potential involvement in cardiovascular and metabolic disease (Talens et al., 2010). The investigators observed extensive inter-individual variation in CpG methylation in blood, with the exception of loci that are either fully methylated or unmethylated. The extent of variation was different from loci to loci. The authors stress that DNA methylation should be considered as a quantitative trait more than just an either/or quality. The high degree of inter-individual variation in DNA methylation for several loci is worth remembering when interpreting small differences in clinical studies.

When investigating epigenetic changes, the researcher has the difficult task of deciding which genomic location to investigate. With the complicated gene structure and multiple promoters of *BDNF* this is not easy and often earlier studied regions are selected. Most studies focus on CpG's in promoters I and IV (Table 2), but intragenic regions may also be important to investigate. The choice is further complicated by the tissue-specific expression pattern that largely depends on promoter usage. Therefore, investigation of epigenetic changes in one promoter in leukocytes may not say much about regulation in the brain. DNA methylation has customarily been studied as the epigenetic mark, because it is considered relatively stable, whereas histone modifications are more transitory (Kofink et al., 2013). At present, there is a growing acknowledgment of the dynamic nature of DNA methylation and that this active epigenetic flexibility can be important in itself (Baker-Andresen et al., 2013). Even though histone modifications may be just as important to investigate, several things are in favor of the use of DNA methylation analysis in epigenetic epidemiology: the amenability to high-throughput analysis and the possibility of using DNA from whole blood samples taken by standard procedure for other purposes, which makes samples more attainable. However, future research is needed to examine the role of 5 hmC in *BDNF* epigenetic regulation, since standard bisulfite treatment for detection of methylated cytosines does not distinguish between 5 mC and 5 hmC (Huang et al., 2010; Jin et al., 2010).

There has been great progress in the technology used to examine DNA methylation levels with the feasibility of high-throughput analysis and genome-wide investigation (Heijmans and Mill, 2012). However, procedures for normalization and quality control have not been standardized and it is a challenge to compare results from different platforms where both individual CpG sites as well as groups of CpG's are measured. In addition, when analyzing loci with intermediate levels of methylation, different methods can result in significant discrepancies in methylation levels and the choice of method is often a compromise between coverage and

resolution. Quality-control and verification procedures for epigenetic studies are recommended (Pidsley and Mill, 2011).

The differences in DNA methylation between psychiatric groups and healthy controls, if present, are often very small (Table 2). It is unknown whether these very small changes have any functional effects on transcription. Unfortunately, there are few studies investigating the effect of epigenetic changes at specific sites on gene transcription in human subjects or in vitro. This has been investigated for *BDNF* in animal and cellular models, showing association between the DNA methylation level in promoters I, IV, and VI and gene transcription (Ikegame et al., 2013a). One study has measured *BDNF* protein in blood in order to study gene expression in human subjects (Perroud et al., 2013). This practice has several challenges as multiple determinants of blood *BDNF* level exist. *BDNF* protein levels are, for example, highly influenced by menstrual cycle, exercise, smoking, and drinking habits (Bus et al., 2011; Cubeddu et al., 2011). In addition, some methods for the detection of *BDNF* levels do not distinguish between *BDNF* and pro*BDNF*, which is a problem because pro*BDNF* has an opposing effect, as it promotes cell death (Lu et al., 2005).

Another issue is that clinical studies may not be hypothesis-driven in regards to how the transcription level of a certain gene can affect neural function. The mere existence of epigenetic changes in a clinical group compared to healthy controls cannot be used to conclude anything about causality. The epigenetic changes can very well be secondary to the intake of medication or disease process, or be caused by confounding factors such as diet, smoking and socioeconomic factors (Heijmans and Mill, 2012; Philibert et al., 2013; Tehranifar et al., 2013; Widiker et al., 2010). Investigation of causality is hampered because it is not possible to carry out longitudinal studies using brain tissue. Often, sample size is very small, which makes it difficult to discover discrete differences in epigenetic markers that might have an effect in transcription. The small samples also hamper the use of genome-wide analyses that demand bigger samples in order to gain statistical significance. Thus, researchers must look at specific regions and this involves the difficulties mentioned above.

Epigenetic changes in psychiatric groups are often investigated by psychologists and psychiatrists that lack understanding of the molecular biological methods used to assess and interpret DNA methylation results. On the other hand, when studies are performed by molecular biologists, there may be a lack of understanding of the diagnostic classification and psychometrics. Furthermore, pressure to publish and high expectations to the field of epigenetics may lead to over-optimistic interpretation of results. To achieve useful and correct results in the field of psychiatric epigenetics, experienced researchers within both psychiatry and molecular genetics must cooperate and overcome differences in research methods and terminology. In addition, reviewers and readers are recommended to be critical about insufficient description of methods and conclusions which are not substantiated.

4. Perspectives for *BDNF* therapy in psychiatry

Accumulating evidence suggests a role for epigenetics in the etiology of complex disorders, including psychiatric disorders

(Labrie et al., 2012). For example, misregulation of epigenetic modifications, also called epimutations, are proposed to explain the phenotypic discordance seen in monozygotic twin studies, as well as some de novo cases (Petronis, 2010). Epimutations may arise from random errors in the maintenance of epigenetic marks or by the impact of environmental factors, and is likely to be affected by cis-acting DNA polymorphisms (Tycko, 2010). Given the importance of *BDNF* during brain development and learning, it is perhaps not surprising that epigenetic changes in this gene have been sought – and found – in human subjects with schizophrenia, depression, bipolar disorder and borderline personality disorder (Table 2). Although suggestive, demonstration of a causal role for these epigenetic changes in the etiology of these psychiatric disorders awaits further study.

In addition to its role as a stable epigenetic modification during early development, DNA methylation is proposed to be an important mechanism in experience-driven changes in the brain (Baker-Andresen et al., 2013). The authors suggest that activity-modified changes in DNA methylation may not affect transcription directly, but rather engender structural changes at the genomic locus that alter the transcriptional response to future stimuli, e.g. by changing the relative expression of alternative splice variants. With regards to the epigenetic regulation of *BDNF*, direct and indirect effects of DNA methylation are potential targets of therapy. In order to reduce non-specific effects, targeted repair of epigenetic misregulation may be achieved through use of small interfering RNAs or by sequence-specific DNA-binding proteins linked to epigenetic modifying enzymes (Labrie et al., 2012).

Finally, accumulating evidence suggests that epigenetic mechanisms are involved in the action of antidepressants, mood stabilizers and psychotherapy at the human *BDNF* gene promoter (Table 2). DNA methyltransferase and histone deacetylase inhibitors, currently being tested in clinical trials for cancer treatment, could have applications in treating psychiatric disorders (Bouille et al., 2012; Gavin et al., 2013). Epigenetic misregulation due to folic acid or vitamin B₁₂ deficiency may be amenable to nutritional therapy (Peedicayil, 2012). Furthermore, epigenetic profiling of *BDNF* is applicable in both diagnosis and in predicting treatment response in depressed patients (Fuchikami et al., 2011; Tadic et al., 2014). Complementary approaches include epigenetic studies of factors involved either in *BDNF* signaling, such as the TrkB receptor itself (Ernst et al., 2009), or in the epigenetic regulation of *BDNF*, including MeCP2 (Li and Pozzo-Miller, 2014).

5. Conclusion

BDNF has a central role in neuronal development and neuroplasticity, and its deregulation is implicated in a range of neurodegenerative and psychiatric disorders. The rapid pace of research in the epigenetic modifications and mechanisms controlling *BDNF* gene expression indicates that progress in *BDNF* epigenetics will have widespread applications in diagnosis, prognosis and biomarkers for psychiatric disorders.

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