Genetic & epigenetic differences between borderline personality disorder patients & healthy controls

FURRER, Sandra

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Reference

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Genetic & epigentic differences between borderline personality disorder patients & healthy controls

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Abstract

**Background:** Borderline personality disorder (BPD) is a complex and serious mental disorder, which risk factors BPD are believed to be both biological and environmental. The BDNF gene is believed to play a pivotal role in mental disorders. Nevertheless, not much is known about the BDNF gene in relation to BPD or about the methylation levels at the BDNF gene in BPD. Moreover, it is not clear how psychotherapy, intensive dialectic behavior therapy (I-DBT) respectively, affects methylation levels in BPD.

**Objective:** The aim of the present study was to compare BPD patients with healthy controls on the score of single nucleotide polymorphisms (rs2030324, rs988748, rs6265, rs7124442) and length polymorphisms (BDNF-LCPR and BDNF microsatellite) on the BDNF gene. Furthermore, BDNF methylation levels (BDNF promoter 4 and BDNF 81B) should be compared between the two groups. An additionally goal was to assess the effect of I-DBT on methylation levels at the BDNF gene.

**Method:** In the present study, 112 patients with DSM-IV BPD diagnosis and 114 healthy controls were taken peripheral venous blood samples. After genomic DNA extraction, the samples were amplified by PCR and afterwards genotyped. For statistical analyses cases-control analyses were conducted.

To assess methylation levels the extracted DNA was bisulfite-modified, amplified and finally the HRM profile was analyzed. For statistical analysis a linear regression considering continuous genotypes and a generalized linear mixed model were used.

**Results:** Genetic differences between BPD patients and healthy controls were found at SNP rs988748 and in the length polymorphism BDNF microsatellite. Furthermore, 29 haplotypes were identified, which are significantly more often present in BPD patients than in healthy controls. Mean methylation levels differ at BDNF promoter 4 and BDNF 81B between BPD patients and healthy controls. Finally, mean methylation levels at BDNF promoter 4 and BDNF 81B differ before and after I-DBT.

**Conclusion:** Summing up, the results suggest that there are genetic as well as epigenetic differences between BPD patients and healthy controls in different regions on the BDNF gene. Moreover, it seems that I-DBT can change methylation levels in BPD patients.
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1. Introduction

1.1. Borderline personality disorder (BPD)

1.1.1. Definition

Borderline personality disorder (BPD) is a complex and serious mental disorder, which is characterised by a marked impulsivity and a pervasive pattern of instability in interpersonal relationships, self-image and affects. It is associated with severe functional impairment, substantial treatment utilization, and a high mortality rate by suicide. Mortality rate in BPD patients is almost 10% to 50% times higher than the rate in the general population. The set of problems is manifested in a broad range of situations and life contexts and usually has its onset by late adolescence or early adulthood. In the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 2004), nine diagnostic criteria for BPD are listed (Table 1), out of which five have to be applied for a BPD diagnosis.

<table>
<thead>
<tr>
<th>Diagnostic criteria for Borderline Personality Disorder</th>
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<tbody>
<tr>
<td>A pervasive pattern of instability of interpersonal relationships, self-image, and affects, and marked impulsivity beginning by early adulthood and present in a variety of contexts, as indicated by five (or more) of the following:</td>
</tr>
<tr>
<td>(1) frantic efforts to avoid real or imagined abandonment</td>
</tr>
<tr>
<td>(2) a pattern of unstable and intense interpersonal relationships characterized by alternating between extremes of idealization and devaluation</td>
</tr>
<tr>
<td>(3) identity disturbance: markedly and persistently unstable self-image or sense of self</td>
</tr>
<tr>
<td>(4) impulsivity in at least two areas that are potentially self-damaging (e.g., spending, sex, substance abuse, reckless driving, binge eating)</td>
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<td>(5) recurrent suicidal behaviour, gestures, or threats, or self-mutilating behaviour</td>
</tr>
<tr>
<td>(6) affective instability due to a marked reactivity of mood (e.g. intense episodic dysphoria, irritability, or anxiety usually lasting a few hours and only rarely more than a few days)</td>
</tr>
<tr>
<td>(7) chronic feelings of emptiness</td>
</tr>
<tr>
<td>(8) inappropriate, intense anger or difficulty controlling anger (e.g., frequent displays of temper, constant anger, recurrent physical fights)</td>
</tr>
<tr>
<td>(9) transient, stress-related paranoid ideation or severe dissociative symptoms</td>
</tr>
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Table 1: DSM-IV: Diagnostic criteria for Borderline Personality Disorder (American Psychiatric Association, 2004).
1.1.2. Epidemiology

Prevalence of BPD in the general population is greater than initially assumed. While Torgersen, Kringlen and Cramer (2001) estimated 1% to 2% of the general population to be affected, Grant et al. (2008) estimated 5.9% to be affected. In most epidemiologic surveys prevalence of BPD does not differ by sex (Coid, Yang, Tyrer, Roberts, & Ullrich, 2006; Grant et al., 2008; Jackson & Burgess, 2000; Lenzenweger, Lane, Loranger, & Kessler, 2007; Torgersen, Kringlen, & Cramer, 2001), but it often does in clinical studies (Widiger, 1998; Widiger & Weissman, 1991).

Clinical studies have shown BPD to be highly comorbid with other psychiatric disorders (Grant, et al., 2008; Oldham et al., 1995; Skodol et al., 1999; Zanarini et al., 1998a, 1998b; Zimmerman & Mattia, 1999). Looking for comorbidities in BPD patients Grant et al. (2008) found similar rates for lifetime and 12-month co-occurrence of other psychiatric disorders, respectively. Highest rates were found for panic disorder with agoraphobia, bipolar disorder I, and drug dependence. A little bit lower, but still high rates were found for mood disorders, anxiety, and substance use disorders (Table 2) (Grant, et al., 2008).

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Lifetime co-occurrence</th>
<th>12-month co-occurrence</th>
</tr>
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<tbody>
<tr>
<td>Panic disorder with agoraphobia</td>
<td>36.0%</td>
<td>51.0%</td>
</tr>
<tr>
<td>Bipolar disorder I</td>
<td>35.9%</td>
<td>55.1%</td>
</tr>
<tr>
<td>Drug dependence</td>
<td>30.9%</td>
<td>45.8%</td>
</tr>
<tr>
<td>Mood disorders</td>
<td>17.2%</td>
<td>29.4%</td>
</tr>
<tr>
<td>Anxiety disorders</td>
<td>14.8%</td>
<td>21.5%</td>
</tr>
<tr>
<td>Substance use disorder</td>
<td>9.5%</td>
<td>14.7%</td>
</tr>
</tbody>
</table>

Table 2: Lifetime and 12-month co-occurrence of other psychiatric disorders in BPD patients (Grant, et al., 2008)

Risk factors for BPD are believed to be both biological and environmental. While some scientists plead for genetic inheritance of neurobiological abnormalities, others focus on a history of adverse experience (Gunderson & Hoffman, 2005).

Lieb, Zanarini, Schmahl, Linehan and Bohus (2004) proposed a complex neurobehavioral model for the cause of BPD (Figure 1). This model involves several factors, which interact in various ways with each other. For example, genetic factors and adverse childhood experiences are considered to be causes for emotional dysregulation and impulsivity. Thus, they have an impact on dysfunctional behaviour, and psychosocial conflicts and deficits. These facts again may reinforce emotional dysregulation and impulsivity (Lieb, Zanarini, Schmahl, Linehan, & Bohus, 2004).
Clinical studies suggest that environmental factors play an important role in the genesis of BPD. 71% of BPD patients report the experience of severe childhood trauma (Lieb, et al., 2004). Various types of childhood adversity and traumatic life events are experienced by many patients. BDP patients showed a significantly higher rate of traumas, such as physical abuse (71%), sexual abuse (68%), and witnessing serious domestic violence (62%) than non-BPD patients (Herman, Perry, & Vanderkolk, 1989). Comparing BDP patients with depressive patients, the former reported a significant higher frequency of sexual abuse than the later (p=0.005) (Ogata et al., 1990). Compared to healthy control subjects, BPD patients experienced significantly more often early traumatic life events including sexual abuse, violence in the family, loss of mother, father or parents, childhood illness, etc. (Bandelow et al., 2005).

Genetic studies, which provide evidence for genetic factors as a cause for BPD, are rare. Notwithstanding, conducting a twin study, Torgersen et al. (2000) found concordance for definite BPD of 35% in monozygotic twin pairs and only 7% in dizygotic pairs. This obviously implies a genetic component in BDP (Torgersen et al., 2000).

1.1.3. Therapy

There are several psychotherapeutic approaches to treat BPD. However, one of the most studied and most efficient cognitive behavioural psychotherapies is the so called dialectic behaviour therapy (DBT) (M. Linehan, 1993a, 1993b). Many authors showed that compared with treatment-as-usual, DBT reduces effectively core symptoms of BDP (Bohus et al., 2004; Bohus et al., 2000; M. M. Linehan, Armstrong, Suarez, Allmon, & Heard, 1991; M. M. Linehan et al., 2006). The standard DBT program was originally developed for outpatients, chronically parasuicidal and meeting criteria for BPD. The hierarchically arranged goals of the DBT are far-reaching. They include (1) the reduction of suicidal behaviour, (2) of behaviours that interfere with treatment delivery and (3) of other dangerous, severe, or destabilizing behaviours. Furthermore, they comprise of (4) acquiring behavioural skills, (5) decreasing posttraumatic stress responses related to previous traumatic events, (6) increasing self-respect and lastly (7) meeting other goals of the patient is comprised. To best reach these goals the following four service modes are provided: (1) weekly individual psychotherapy for 1 hour per week, (2) 2½ hours group skills training per week, (3) telephone consultation (as needed within the therapist’s limits to ensure generalization), and (4) weekly therapist consultation team meetings to enhance therapist motivation and skills (M. Linehan, 1993a, 1993b; M. M. Linehan, et al., 2006).
McQuillan et al. (2005) have developed a shortened intensive version of the standard DBT. The intensive dialectical behaviour therapy (I-DBT) was found to be an effective treatment, which offers the essential components of the standard DBT within 3 weeks: individual and group therapy and limited phone call availability of the therapists between 8:30 a.m. and 6:00 p.m. (McQuillan et al., 2005).

### 1.2. Gene-environment interaction

Nowadays, the gene-environment interaction approach is often used in psychiatric research, since not only genes or the environment are considered to be a cause for mental psychiatric disorders. Gene-environment interaction occurs when the effect of exposure to an environmental risk on health is conditional on a person’s genotype or inversely, when environmental experience moderates genes’ effects on health.

While some individuals exposed to environmental pathogen develop mental disorders, some others exposed to the same environmental pathogen do not develop it. This implies differences in genetic susceptibility, which are involved in the outbreak of the mental disorders.

Stress, more precisely traumatic events, is a well examined environmental factor and seems to play a pivotal role in mental disorders. They are assumed to initiate and exacerbate mental disorders such as depression (Brown, 1998; Burnam et al., 1988; Kendler, Karkowski, & Prescott, 1999; Kessler, 1997; Pine, Cohen, Johnson, & Brook, 2002), post-traumatic stress disorder (PTSD) (Breslau, Davis, Andreski, & Peterson, 1991; Iteke, Bakare, Agomoh, Uwakwe, & Onwukwe, 2011), panic disorder (Goodwin, Fergusson, & Horwood, 2005), anxiety disorders (Burnam, et al., 1988), obsessive-compulsive disorders (OCD) (Lochner et al., 2002), eating disorders (Rayworth, Wise, & Harlow, 2004), and substance abuse and dependence (Burnam, et al., 1988).

Up to now, most studies investigated the relation between stress and serotonin (Caspi et al., 2003; Frodl et al., 2010), because the hypothalamic-pituitary-adrenocortical (HPA) axis is the key hormonal component of stress responses (Chaouloff, 1993). Nevertheless, stress not only affects the serotonergic system, but also the expression of the brain-derived-neurotrophic factor as it had been shown by different authors. Acute and chronic stress alters the expression of *BDNF*. In detail, stress decreases *BDNF* mRNA levels in the mouse and rat brain (Nibuya, Takahashi, Russell, & Duman, 1999; Pizarro et al., 2004; Schaaf, de Jong, de Kloet, & Vreugdenhil, 1998; Smith, Makino, Kvetnansky, & Post, 1995; Vaidya, Marek, Aghajanian, & Duman, 1997; Vaidya, Terwilliger, & Duman, 1999). To examine the association of BDNF levels and lifetime trauma in patients with bipolar disorder, venous blood was taken from such patients. As a result, BDNF levels were found to be significantly decreased in bipolar patients with a history of trauma, compared to patients without a history of trauma (p=0.002)(Kauer-Sant'Anna et al., 2007).

Further studies focused on polymorphisms of the *BDNF* gene. Perroud et al. (2007) conducted a study, examining the effect of the interaction between the *BDNF* gene and childhood maltreatment on suicidal behaviour (SB). Summarizing the results, the polymorphism *Val66Met* modulates the effect of childhood sexual abuse on the violence of SB. In more detail, among Val/Val individuals childhood sexual abuse was associated with violent suicide attempts in adulthood only, but not among *Val/Met* or *Met/Met* individuals (p=0.05). Moreover, the more severe the childhood maltreatment, the higher the number of suicide attempts and the lower the age at onset of suicide attempts (Perroud et al., 2007).
Another study also found BDNF-environment interactions. Kim et al. analysed the relation of the BDNF gene, stressful life events and depression in Korean child, adolescent, and adult populations. While the association between stressful life events and depression was significant in Met/Met homozygous patients (p=0.007) and Val/Met heterozygous patients (p=0.023), it was not in Val/Val homozygous ones (p=0.967). These results suggested that environmental risk of depression, in a context of stressful life events, is modified by the BDNF gene, even into old age (Kim et al., 2007).

Taken together, BDNF polymorphisms and BDNF gene expression play an important role for several psychiatric disorders in interaction with traumatic experiences. Nevertheless, the mechanism leading to this downregulation of BDNF expression in humans is up to now poorly understood.

1.3. Epigenetics

Epigenetic pathways could be involved in the dysregulation of BDNF gene expression. Epigenetics leads to variations in gene expression caused by mechanisms other than changes in the underlying DNA sequence. A major epigenetic mechanism is DNA methylation. In this case, changes in the gene expression are caused by covalently linked (CH3) groups to cytosine molecules. DNA methylation typically, but not only, occurs at CpG sites. CpG sites, also called CpG islands, are cytosine-phosphate-guanine sites, where a cytosine is directly followed by a guanine in the DNA sequence. (Gruenbaum, Stein, Cedar, & Razin, 1981). Evidence shows that if cytosines are methylated in the gene promoter, these covalent modifications can affect gene transcription by altering the accessibility of RNA polymerase and transcription factors (Jaenisch & Bird, 2003).

DNA methylation of the BDNF gene is progressively suggested to mediate gene-environment interplay throughout the lifespan and is considered to be both dynamic (Lubin, Roth, & Sweatt, 2008; Yossifoff, Kisliouk, & Meiri, 2008) and stable (Onishchenko, Karpova, Sabri, Castren, & Ceccatelli, 2008; Sweatt, Roth, Lubin, & Funk, 2009) alterations in the CNS (Sweatt & Roth, 2011).

In an animal model, Roth, Zoladz, Sweatt and & Diamond (2011) showed that rats, exposed to psychosocial stress, have significantly increased BDNF DNA methylation in the dorsal hippocampus, with the most robust hypermethylation detected in the dorsal CA1 subregion. Inversely, the psychosocial stress regimen significantly decreased methylation in the ventral hippocampus (CA3). No changes in BDNF DNA methylation were detected in the medial prefrontal cortex or basolateral amygdala (Roth, Zoladz, Sweatt, & Diamond, 2011). These results proofed that traumatic stress can induce CNS gene methylation. However, these results cannot be directly transferred to humans as an animal model was used in this study.

Based on their data, Tsankova et al. (2006) suggested a model by which chronic stress induces repression of the BDNF gene in the hippocampus in mice. Whereas the BDNF gene is expressed at some basal level under normal conditions, chronic defeat stress induces dimethylation at the BDNF gene, which persists long after the end of stress. Compared with methylation, where two methyl groups are added to a molecule, dimethylation means the addition of two methyl groups to the molecule (Tsankova et al., 2006).

Keller et al. (2010) investigated brain samples from suicide subjects postmortem. Methylation and BDNF mRNA levels were assessed. Results showed a statistically significant increase of DNA methylation at specific CpG sites in BDNF promoter IV compared with non-suicidal control subjects. Additionally, the data suggested that increased DNA methylation levels of BDNF promoter IV can negatively regulate BDNF expression in suicide subjects as well as controls (Keller et al., 2010).

Despite all the research presented above, no data on the methylation status of BDNF in humans suffering from BPD exists.
1.4. Pharmacogenetics

Pharmacogenetics, a continuative field of studies, combines gene-environment interaction and pharmacology. Genetics influences therapeutic response in patients with psychiatric disorders, as showed by several research groups (Eisenegger et al., 2010; Perroud et al., 2010; Uher et al., 2009; Uher et al., 2010). Nonetheless, treatments and their response are poorly understood on that score and not many studies exist. Most of the existing studies investigate the effect of antidepressants on depression, but no other pharmaceutical treatments dispensed for sundry psychiatric disorders as for example BPD. Furthermore, studies are missing, which investigate non-pharmaceutical treatment forms, such as psychotherapy. This poses a serious issue, since the lack of efficacy in many of the patients together with side effects can both limit therapy and compliance (Rujescu & Moller, 2010).

1.5. Candidate gene: brain-derived-neurotrophic-factor gene

The brain-derived-neurotrophic-factor gene, lying on chromosome 11p14.1, encodes the homonymous protein brain-derived-neurotrophic-factor. This protein plays an important role in the development, regeneration survival and maintenance of functions of neurons (Maisonpierre et al., 1990). This factor responsible for the modulation of synaptic plasticity and neurotransmitter release across different neurotransmitter systems (Thoenen, 1995).

BDNF variations and BDNF mRNA levels are found to be relevant to several psychiatric disorders: bipolar disorder (Geller et al., 2004; Neves-Pereira et al., 2002; Sklar, Gabriel, et al., 2002; Sklar, Smoller, et al., 2002), obsessive compulsive disorder (Estivill et al., 2008; Karayiorgou, Hall, Dhilla, Charalambous, & Gogos, 2003) and schizophrenia (Kennedy et al., 2003; Nawa et al., 2000; Weickert et al., 2003).

Licinio, Dong and Wong (2008) resequenced in patients with major depressive disorder and in controls a genomic DNA region of 22 kilobase that included all BDNF exons and their flanking regions. As a result they identified 83 novel SNPs. Out of those 83 SNPs, 6 were associated with major depressive disorder (MDD) (Licinio, Dong, & Wong, 2009).

As Licino et al. have shown, extensive resequencing of key candidate genes can lead to the discovery of substantial numbers of variants, which might be associated with a specific psychiatric disorder and treatment response.

1.5.1. BDNF promoter 4

BDNF promoter 4 is localized at position: 27723145–27723086 bp on the BDNF gene and has a size of 59 bp (http://genome.ucsc.edu/cgi-bin/hgGateway, February 2009 (GRCh37/hg19), 2011). It contains 4 CG sites and seems to be crucial during development. Evidence has been provided that BDNF promoter 4 is highly regulated during the development of the forebrain in mice, and DNA methylation plays a key role in this regulation (Dennis & Levitt, 2005).

In addition to this, it has been shown in mice brain tissue that after chronic defeat stress there is a strong increase in dimethylation at BDNF pormoter 4 (Tsankova, et al., 2006). The same effect was observed in human brain tissue in the Wernicke area of suicide subjects. In other words, BDNF promoter 4 often is hypermethylated in the Wernicke area of the postmortem brain of suicide subjects (Keller, et al., 2010).
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1.5.2. **CpG 81B**

CpG 81 is located on chromosome 11p14.1 at position: 27700049–27701140 bp on the *BDNF* gene and has a total size of 1092 bp. CpG 81B is a part of CpG 81 and counts 15 CG sites. Its size amounts to 315 bp ([http://genome.ucsc.edu/cgi-bin/hgGateway](http://genome.ucsc.edu/cgi-bin/hgGateway), February 2009 (GRCh37/hg19), 2011)

1.5.3. **BDNF microsatellite**

This microsatellite is defined as a sequence of 16 dinucleotide repeats of GT. It is located at position 27695984–27696015 bp near *BDNF* gene promoter, in band 11p14.1. Its genomic size amounts to 32 bp ([http://genome.ucsc.edu/cgi-bin/hgGateway](http://genome.ucsc.edu/cgi-bin/hgGateway) February 2009 (GRCh37/hg19), 2011).

1.5.4. **rs2030324**

These single nucleotide polymorphisms (SNP), which can either be C or T. It is located at position 27683491–27683491 bp on the *BDNF* gene ([http://genome.ucsc.edu/cgi-bin/hgGateway](http://genome.ucsc.edu/cgi-bin/hgGateway), February 2009 (GRCh37/hg19), 2011).

1.5.5. **rs988748**

This SNP can either be C or G. It is located at position 27681321-27681321 bp, in band 11p14.1 ([http://genome.ucsc.edu/cgi-bin/hgGateway](http://genome.ucsc.edu/cgi-bin/hgGateway), February 2009 (GRCh37/hg19), 2011).

1.5.6. **BDNF-linked complex polymorphic region (BDNF-LCPR)**

As said by its name, the *BDNF*-linked complex polymorphic region (*BDNF*-LCPR) is a region on the *BDNF* with a genomic size that varies ([http://genome.ucsc.edu/cgi-bin/hgGateway](http://genome.ucsc.edu/cgi-bin/hgGateway), February 2009 (GRCh37/hg19), 2011).

Okada et al. (2006) obtained evidence approving the association between *BDNF*-LCPR and bipolar disorder. They investigated a region located about 1.0 kb upstream of the translation initiation site of the *BDNF* gene (Figure 2a). They were looking for polymorphisms, association with bipolar disorder, and effects on transcriptional activity. Detailed sequencing uncovered a region on the *BDNF* gene, which is highly polymorphic and not only a simple dinucleotide repeat. It is a complex structure comprising three types of dinucleotide repeats, insertion/deletion, and nucleotide substitutions (Figure 2b) that gives rise to a total of 23 novel allelic variants. (Okada et al., 2006).
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Figure 2: a. Schematic illustration of the BDNF gene with its coding region and non-coding exons.
b. Schematic illustration of the BDNF-LCPR. (Okada, et al., 2006).

Since BPD patients often suffer from bipolar disorder (Grant, et al., 2008), polymorphisms in the BDNF-LCPR might also play a pivotal role in BPD.

1.5.7. rs6265 (Val66Met)

This SNP can either be G or A. Mean allele frequency in human beings for G amounts to 76.542%, allele frequency for A to 23.458%. It is located at position 27636492 bp, in band 11p14. (http://genome.ucsc.edu/cgi-bin/hgGateway, February 2009 (GRCh37/hg19), 2011).

1.5.8. rs7124442

This SNP can either be T or C. Allele frequency for T amounts to 67.875%, allele frequency for C to 32.125%. It is located at position 276336171 bp (http://genome.ucsc.edu/cgi-bin/hgGateway, February 2009 (GRCh37/hg19), 2011).
Despite all the studies which have been conducted until now, it is still not clear, how polymorphisms and methylation levels on BDNF gene in BPD behave. Moreover, it is not known, what kind of impact treatment on the methylation status has. To address these questions the following hypotheses were formulated and the study in hand was conducted.

### 1.6. Hypotheses

#### 1.6.1. H1: Genotypic differences between BPD patients & healthy subjects

The first hypothesis supposes genotypic differences on the BDNF gene in two regions (BDNF-LCPR and BDNF microsatellite) and 4 SNPs (rs2030324, rs988748, rs6265, rs7124442), comparing BPD patients and healthy subjects.

#### 1.6.2. H2: Differences in DNA Methylation between BPD patients & healthy subjects

The second hypothesis supposes differences in DNA methylation levels on the BDNF in two regions (BDNF promoter 4 and BDNF 81B), comparing BPD patients and healthy subjects.

#### 1.6.3. H3: Differences in DNA Methylation in BPD patients between T1 & T2

The third hypothesis supposes differences in DNA methylation levels on the BDNF gene in two regions (BDNF promoter 4 and BDNF 81B) in BPD patients before Intensive Dialectical Behaviour Therapy (T1) and after Intensive Dialectical Behaviour Therapy (T2).

#### 1.6.4. H4: Genotypic influence on therapeutic response (methylation levels)

The forth hypothesis supposes a modulation of the methylation levels on the BDNF gene in two regions (BDNF promoter 4 and BDNF 81B) in BPD patients and healthy subjects by genetic differences (rs2030324, rs988748, rs6265, rs7124442).
2. Material & methods

2.1. Participants

A total of 112 patients with DSM-IV BPD diagnosis (6 males and 106 females, mean age ± SD = 30.7 ± 9.6 years) and 114 healthy controls were recruited for this study. All patients took part in the Program CAIRE, a program especially created of the Hospital of Geneva for outpatients with BPD who are in crisis. Patients and control subjects gave their written informed consent to participate in the study after a complete and extensive description. Participants were included if they met DSM-IV criteria for BPD. Comorbidity and medication intake was not controlled nor was ethnicity, since the present work presents preliminary work for later research.

2.2. Clinical assessment

For clinical assessment the following questionnaires was used:

The Diagnostic Interview for Genetic Studies (DIGS) was specifically developed for genetic studies. It has polydiagnostic capacities and allows a detailed assessment of the course of the illness and the chronology of affective disorder, psychotic disorders and comorbidities. Moreover, an additional description of symptoms including the possibility of an algorithmic scoring is possible. The DIGS also includes a section to assess temporal relationships between affective disorders, anxiety disorders, psychosis and substance abuse disorders. (Nurnberger et al., 1994)

The Beck Depression Inventory (BDI) is a self-report inventory, consisting of 21 multiple choice questions. It is one of the most widely used instruments for measuring the intensity of depression. (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961). In this study the BDI-II, a revision of the original BDI, was applied (Beck, Steer, Ball, & Ranieri, 1996).

The International Personality Disorder Examination (IPDE) is a semi-structured clinical interview, which allows assessing personality disorders and making diagnoses in accordance with both ICD-10 and DSM-IV criteria. It consists of ten subscales, each one corresponding to a personality disorder. In general, a score greater than 3 on a subscale is necessary to indicate the presence of a personality disorder such as borderline (Loranger, Janca, & Sartorius, 1997).

2.3. Procedure

All patients had to complete the questionnaires listed above. Afterwards, diagnoses were made. If the patients fulfilled the criteria for inclusion the I-DBT was started, comprising of the components described in 1.3. Blood samples of BPD patients were taken shortly before they started the I-DBT (T1) lasting 4 weeks and after they accomplished the therapy. Healthy controls only were taken blood once.

2.4. Genotyping

Genomic DNA was extracted from peripheral venous blood using standard techniques and the Nucleon kit (Bioscience Amersham, GE Healthcare, Glatbrugg, Switzerland). Applying polymerase chain reaction (PCR), two DNA length variations (BDNF microsatellite and BDNF-LCPR) and 4 SNPs (
rs2030324, rs988748, rs6265, and rs7124442) on the BDNF gene were amplified. 5'-TCCACACAGTTTAAGTGAACC-3' (forward primer target) and 5'-TCAAAAAGTGTCAGTCATCTCTC-3' (reverse primer target) was used as primer for the BDNF microsatellite. 5'-TTTTAAAGTAGGATAAACCTCAGAGC-3' (forward primer target) and 5'-TCCCTGCTTTTTTCTGGCTA-3' (reverse primer target) was used as a primer for the BDNF-LCPR. 5'-TCCACACACACAGCGCTTAA-3' (forward primer target) and 5'-TGCTCAAAGGGATGTGAGA-3' (reverse primer target) was used as a primer for rs2030324. 5'-GAACCAACGCAGGGTCT-3' (forward primer target) and 5'-GCAGGCTAACCAGAAAGCAA-3' (reverse primer target) was used as a primer for rs988748. 5'-GAGGCTTGACATCATTGGCT-3' (forward primer target) and 5'-CTGATGAAATGCTTGGAATATCTGC-3' (reverse primer target) was used as a primer for rs6265. 5'-AAGGAATGCTTGGAATATCTGC-3' (forward primer target) 5'-TTGTGCCCTCAAAAAAGGAAGC-3' (reverse primer target) was used as a primer for rs7124442 (Table 3). PCR cycles were performed in the thermocycler.

Target sequences were amplified in a 25 μl reaction solution containing 100 ng genomic DNA, 1x Buffer, 0.12 mM dNTPs, 15 mM MgCl₂, and 0.4 μM of each primer and 1 U Taq polymerase (Eurobio, Brunschwig, Basel Switzerland). Thirty cycles were performed by a Hybaid thermocycler, each consisting of 94°C for 30 s, 54°C for 30 s, and 72°C for 30 s.

<table>
<thead>
<tr>
<th>Marker</th>
<th>forward primer</th>
<th>reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF microsatellite</td>
<td>5'-TCCACACAGTTTAAGTGAACC-3'</td>
<td>5'-TCAAAAAGTGTCAGTCATCTCTC-3'</td>
</tr>
<tr>
<td>rs2030324</td>
<td>5'-TCCACACACACACAGCGCTTAA-3'</td>
<td>5'-TGCTCAAAGGGATGTGAGA-3'</td>
</tr>
<tr>
<td>rs988748</td>
<td>5'-GAACCAACGCAGGGTCT-3'</td>
<td>5'-GCAGGCTAACCAGAAAGCAA-3'</td>
</tr>
<tr>
<td>BDNF-LCPR</td>
<td>5'-TCCCTGCTTTTTTCTGGCTA-3'</td>
<td>5'-TCCACACAGCGCTTAA-3'</td>
</tr>
<tr>
<td>rs6265</td>
<td>5'-GAGGCTTGACATCATTGGCT-3'</td>
<td>5'-CTGATGAAATGCTTGGAATATCTGC-3'</td>
</tr>
<tr>
<td>rs7124442</td>
<td>5'-AAGGAATGCTTGGAATATCTGC-3'</td>
<td>5'-TTGTGCCCTCAAAAAAGGAAGC-3'</td>
</tr>
</tbody>
</table>

Table 3: Primers used to genotype 2 length polymorphisms and 4 SNPs.

PCR products were separated by electrophoresis on a 10% polyacrylamide gel stained with ethidium bromide. Electrophoresis was carried out at 250 V for 7h.

2.5. Methylation

High resolution melting analysis (HRM) is a rapid, cost-effective and reliable method for detection of methylation and SNP genotyping. After bisulfite treatment and PCR reaction, methylated and unmethylated DNA acquire distinctly different melting profiles. While a full methylated allele has a high melting temperature, a less methylated allele has a low melting temperature. Thus, the composition of the HRM profile reflects the build-up of the methylated and unmethylated alleles present in the DNA samples (Wojdacz & Dobrovic, 2007; Wojdacz, Dobrovic, & Algar, 2008).

In the present study, HRM was used to analyse the methylation level within the intragenic region of BDNF promoter 4 and BDNF 81B. First of all, extracted DNA samples from control subjects and patients were bisulfite-modified using the Epitec Bisulfit Kit: conversion and cleanup of DNA for methylation analysis (QIAGEN GmbH, Hilden, Germany) as described by the producer’s handbook. Treatment of DNA with bisulfite converts non-methylated cytosines to uracil, but leaves 5-methylcytosine residues unaffected. In other words, bisulfite treatment introduces specific changes in the DNA sequence, depending on the methylation status of each cytosine. As a result, PCR
products resulting from a template that was originally unmethylated will have a lower melting point than those derived from a methylated template. To create a range of references samples (0%, 2.5%, 5%, 7.5%, 10%, 15%, 20%, 25%, 50%, 75%, 100%) Universal methylated and unmethylated DNA (Chemicon, Temecula, CA) were mixed and bisulfite-modified by the same process as described above.

In a second step, the bisulfite-modified DNA samples were amplified by PCR. PCR reaction was carried out with 80 ng of genomic DNA using the Kappa 2 G Robust Hot Start Kit (Kappa Biosystem) in a final volume of 20 μl containing 1x buffer A (Kappa Biosystem, Cape Town, South Africa), 0.02 mM dNTPs, 7.5 μM of each primer, 0.01 mM Hot Start polymerase and 0.04 μM EvaGreen fluorescent intercalating dye (Invitrogen, Eugene, OR, USA). 5'-TGTGTTGATTTGATT-3' (forward primer target) and 5'-ACTCACACCCCATCAACA-3' (reverse primer target) was used as primer for the BDNF promoter 4. 5'-TGCGTATCGGGGTGTTAAT-3' (forward primer target) and 5'-CGAAACCAACCCCAACATTT-3' (reverse primer target) was used as primer for the BDNF 81B. Amplification conditions were: 95°C for 3 min, 45 cycles of 95°C for 10 s, 60°C for 30 s and 72°C for 10 s.

PCR reaction was followed by HRM analysis Rotor-Gene 6000 instruments (Corbett Life Science, Australia), determining methylation status of all samples, set up in duplicates. It was performed at the temperature increasing from 68°C to 90°C, with the temperature rising by 0.2 °C per second. In this way, percentage of methylation of each sample was evaluated by comparing its HRM profile with HRM profile of standards of methylation described above.

2.6. Statistical analyses

2.6.1. Genotyping

Association analyses of the BDNF gene were performed. Allele and genotype comparisons between BPD patients and controls were conducted using maximum likelihood inference as applied in Unphased (Dudbridge, 2008). This was mainly used because it allows analysis of multi-allelic markers. Since we compared genotypic and allelic distributions of six polymorphic markers between controls and cases, a correction for multiple testing was necessary. The highly conservative Bonferroni correction was used. Thus, after Bonferroni correction p =0.05/6 = 0.0083 was used as threshold for significance.

In the context of the case-control analyses, haplotype analysis were conducted as well, using again the maximum likelihood inference. A minimum of two markers can explain all genetic variations in patients and controls samples.

Linkage disequilibrium (LD) was additionally calculated as D' values for all SNP pairs in healthy controls and in patients with BPD. The presence of Hardy–Weinberg equilibrium in genotype distribution was examined by using the χ²-test for goodness of fit.

Statistical power to detect associations was estimated using the Genetic Power Calculator (http://pngu.mgh.harvard.edu/purcell/gpc/). Therefore, we determined that our BPD sample had 83% power to detect a risk genotype AA with 30% frequency, as controls, and a genotype relative risk for heterozygotes of 2, for homozygotes of 3 and using an additive genotype model at a level = 0.05.
2.6.2. Methylation

All methylation analyses were calculated by STATA (Version 10). To compare the mean methylation status of BPD patients and controls a t-test for independent samples was conducted.

A linear regression considering continuous genotypes was made, to identify if the SNPs modulate the methylation status in BPD patients and controls.

To contrast the mean methylation status of T1 and T1 a generalized linear mixed model was used.

3. Results

3.1. Genotyping

3.1.1. Allelic & genotypic associations

Case-control-study of the BDNF was done for SNPs- and microsatellite-based on allelic and genotypic associations in BPD patients and healthy controls. SNP-based allelic associations showed that out of 4 tested SNPs only one, namely rs988748, is significantly associated with BPD ($p=2.306 \times 10^{-8}$) (Table 4). The allele C was more present in BPD patients (0.8382) than in controls (0.5922), in contrast the allele G has a higher frequency in controls (0.4078) than in BDP patients (0.4078). The odds ratio amounts to 3.568. To sum up, allele G seems to be protective for BPD, while allele C seems to favour BPD.

SNP-based genotypic associations, showed that out of 4 tested SNPs again SNP rs988748 is significantly associated with BPD ($p=5.605 \times 10^{-5}$) (Table 4). In more detail, the genotype C / C is significantly more present in BPD patients (0.6765) than in controls (0.3883) ($p=1.778 \times 10^{-5}$), in contrast the genotype G / G has a significant higher frequency in controls (0.2039) than in BDP patients (0) ($p=9.631 \times 10^{-5}$). The odds ratio amounts to 3.293. To sum up, genotype G / G seems to be protective for BPD, while allele C / C seems to favour BPD.

<table>
<thead>
<tr>
<th>Marker</th>
<th>p-Value</th>
<th>Allele/ Genotype</th>
<th>Case</th>
<th>Control</th>
<th>Case-Frequency</th>
<th>Control-Frequency</th>
<th>p-Value</th>
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<tbody>
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<td>0.7011</td>
<td>C</td>
<td>88</td>
<td>86</td>
<td>0.4536</td>
<td>0.4343</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
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<td>0.7018</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td></td>
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<td>0.4646</td>
<td>0.4767</td>
</tr>
<tr>
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<td></td>
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<td>122</td>
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<td>0.5922</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>G</td>
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<td>84</td>
<td>0.1618</td>
<td>0.4078</td>
<td>1.356 $\times 10^{-8}$</td>
</tr>
<tr>
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<td>5.605 $\times 10^{-5}$</td>
<td>C / C</td>
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<td>G / G</td>
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<td></td>
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<td>38</td>
<td>0.2816</td>
<td>0.3423</td>
<td>0.3361</td>
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Analyses of microsatellite-based allelic associations showed that BDNF microsatellite is significantly associated with BPD ($p=3.064 \times 10^{-5}$), but not BDNF-LPCR ($p=0.253$) (Table 5). In more detail, the allele 104, located at the BDNF microsatellite, is significantly more present in controls (0.1311) than in BPD patients (0.02062) ($p=2.172 \times 10^{-6}$). On the contrary, the allele 106, located at the BDNF microsatellite, is significantly more often present in BPD patients (0.04124) than in controls (0.004854) ($p=2.046 \times 10^{-6}$). The odds ratio amounts to 8.473. Allele 92 might also be significantly different distributed among cases (0) and controls (0.0097089). However, since only 2 individuals in the control group showed the allele 92 and 0 individuals of the BPD group, the $p$-value is not valuable. In summary, while allele 104 seems to be protective for BPD, allele 106 seems to favour BPD. Allele 92 is a potential protective factor for BPD. Nevertheless, this has to be retested in an enlarged sample.

In the length polymorphism BDNF-LPCR, the alleles 124, 130, and 134 might also be significantly different distributed among cases and controls. However, since not enough individuals in the two groups showed the characteristic, the $p$-values are not valuable.

Analyses of microsatellite-based genotypic associations showed that again the BDNF microsatellite is significantly associated with BPD ($p=8.487 \times 10^{-6}$), but not BDNF-LPCR ($p=0.1172$) (Table 5). In addition, several genotypic combinations (94 / 100, 98 / 102, 100 / 102) were found to be significant, although the single alleles of the combinations were not significant themselves.

Even though several allelic combinations of the BDNF-LPCR were significant, they are not meaningful, taking into account the little frequencies of cases and controls.

<table>
<thead>
<tr>
<th>Microsatellite</th>
<th>p-Value</th>
<th>Allele</th>
<th>Case</th>
<th>Control</th>
<th>Case-Frequency</th>
<th>Control-Frequency</th>
<th>p-Value</th>
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<td>92</td>
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<tr>
<td></td>
<td></td>
<td>94</td>
<td>7</td>
<td>5</td>
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<tr>
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<td>96</td>
<td>43</td>
<td>51</td>
<td>0.2216</td>
<td>0.2476</td>
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<td></td>
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<td>64</td>
<td>72</td>
<td>0.3299</td>
<td>0.3495</td>
<td>0.698</td>
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<tr>
<td></td>
<td></td>
<td>100</td>
<td>33</td>
<td>20</td>
<td>0.1701</td>
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<tr>
<td></td>
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<td>102</td>
<td>35</td>
<td>20</td>
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<td>0.174</td>
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<td>104</td>
<td>4</td>
<td>27</td>
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<td>0.1311</td>
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<td></td>
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<td>106</td>
<td>8</td>
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<td>0.04124</td>
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<td>$8.487 \times 10^{-6}$</td>
<td>92 / 96</td>
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<td>1</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>92 / 100</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.009709</td>
<td>3.729 $\times 10^{-6}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>94 / 100</td>
<td>3</td>
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<td>0.03093</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>96 / 104</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.009709</td>
<td>3.729 $\times 10^{-6}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>96 / 106</td>
<td>1</td>
<td>0</td>
<td>0.01031</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>98 / 102</td>
<td>19</td>
<td>6</td>
<td>0.1959</td>
<td>0.05825</td>
<td>8.353 $\times 10^{-6}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>98 / 104</td>
<td>2</td>
<td>16</td>
<td>0.02062</td>
<td>0.1553</td>
<td>1.772 $\times 10^{-6}$</td>
</tr>
</tbody>
</table>
Genetic & epigenetic differences between borderline personality disorder patients & healthy controls

Comparing two-markers combinations of BPD patients and controls, 29 haplotypes were found to differ significantly between the two groups (Table 6). Most significant combinations were found for BDNF microsatellite and rs988748. While some haplotypes seem to be protective (a), others seem to favour (b) BPD.

<table>
<thead>
<tr>
<th>Marker</th>
<th>p-Value</th>
<th>Haplotypes</th>
<th>Cases</th>
<th>Controls</th>
<th>Cases-Frequency</th>
<th>Controls-Frequency</th>
<th>P-value</th>
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<tr>
<td>rs2030324</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF microsatellite</td>
<td>1.23 E-6</td>
<td>102-T (a)</td>
<td>29.82</td>
<td>8.136</td>
<td>0.162</td>
<td>0.04374</td>
<td>6.980 E-3</td>
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<tr>
<td></td>
<td></td>
<td>104-T (b)</td>
<td>2.903</td>
<td>22.7</td>
<td>0.01578</td>
<td>0.1221</td>
<td>5.316 E-3</td>
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</table>

Table 5: Allelic and genotypic associations of microsatellites in BPD patients and controls. Significant values are in bold. BDNF microsatellite: 92 corresponds to (GT)14, 94 corresponds to (GT)15, 96 corresponds to (GT)16, 98 corresponds to (GT)17, 100 corresponds to (GT)18, 102 corresponds to (GT)19, 104 corresponds to (GT)20, 106 corresponds to (GT)21. BDNF-LPCR: each allele corresponds to a size of PCR.

3.1.2. Haplotype-based analyses of BPD patients and controls
<table>
<thead>
<tr>
<th></th>
<th>106-T</th>
<th>8</th>
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<th>0.04348</th>
<th>0.005376</th>
<th>3.031 E^{-4}</th>
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<tbody>
<tr>
<td>rs988748</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>BDNF microsatellite</td>
<td>1.196 E^{-6}</td>
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<tr>
<td>98-G</td>
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<td>0.01048</td>
<td>0.1479</td>
<td>1.632 E^{-5}</td>
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<td>100-C</td>
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<td>0.1719</td>
<td>0.07349</td>
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<td>0.09502</td>
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</tr>
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<td>0.005263</td>
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<td>104-144</td>
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<td>21.93</td>
<td>0.02083</td>
<td>0.1119</td>
<td>1.023 E^{-4}</td>
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Genetic & epigenetic differences between borderline personality disorder patients & healthy controls

<table>
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Table 6: Haplotype-based analyses of BPD patients and controls. Significant values are in bold.

### 3.1.3. Linkage disequilibrium

Linkage disequilibrium (LD) was calculated as D’ values for all SNPs pairs in healthy controls and in patients with BPD (Table 7). Markers are not equally linked in the two groups. Whereas no markers are linked in the healthy control group, the markers rs988748 and rs7124442 and markers rs6265 and rs7124442 are linked (Global D’ value: 1) in BPD patients.

<table>
<thead>
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<th>Global D’ value for polymorphism pair combinations in healthy controls</th>
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<table>
<thead>
<tr>
<th>Global D’ value for polymorphism pair combinations in BPD patients</th>
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<tr>
<td>BDNF microsatellite</td>
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<tr>
<td>rs6265</td>
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<td>rs7124442</td>
</tr>
</tbody>
</table>

Table 7: Linkage disequilibrium (LD)
3.2. Methylation

3.2.1. Comparing BDP vs. controls

3.2.1.1. BDNF promoter 4

Comparing the average percentage of methylation of BPD patients with the average percentage of methylation of controls at BDNF promoter 4, a significant difference between the two groups \((p = 0.00002161)\) was shown (Figure 4). While patients displayed a relatively high methylation status on average (35.82\%, SD ± 27.1\%), controls exhibited a markedly lower mean methylation status (14.86\%, SD ± 13.82\%).

![Box plot showing difference of mean methylation level at the BDNF promoter 4 between BPD and controls.](image)

**Figure 4:** Difference of mean methylation level at the BDNF promoter 4 between BPD and controls.

3.2.1.2. BDNF 81B

Comparing the average percentage of methylation between BPD patients and controls at region BDNF 81B, uncovers also a further significant difference between the two groups \((p = 0.000000464)\) was found (Figure 5). While the patients showed a relatively high methylation status on average (13.18\%, SD ± 12.03\%), controls displayed a markedly lower mean methylation status (2.79\%, SD ± 1.21\%).
3.2.2. Comparing T1 vs. T2 in BPD

3.2.2.1. **BDNF promoter 4**

Mean methylation levels at *BDNF* promoter 4 of BPD patients differed significantly between the date before CBT-I (T1) and after the date of CBT-I (T2) (*p*=0.002) (Figure 6). BPD patients showed a lower methylation level at T1 (35.82%, SD ± 27.02%), in contrast with T2 (47.08%, SD ± 32.10%).

Figure 6: Difference of mean methylation level at the *BDNF* promoter 4 at T1 and T2.
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### 3.2.2.2. BDNF 81B

Mean methylation levels at the region *BDNF* 81B of BPD patients differed significantly between the date before CBT-I (T1) and after the date of CBT-I (T2) \( (p = 0.03) \) (Figure 7). BPD patients at T1 showed a lower methylation levels (13.18%, SD ± 12.03%), comparing to T2 (17.14%, SD ± 11.24%).

![Box plot showing difference in mean methylation levels at BDNF 81B at T1 and T2](image)

**Figure 7:** Difference of mean methylation level at the *BDNF* 81B at T1 and T2.

### 3.2.3. Modulatory effects of SNPs on methylation levels in BPD and controls

#### 3.2.3.1. BDNF promoter 4

No significant modulatory effect was identified, testing markers rs2030324, rs988748, rs6265 and rs7124442 for modulation on the methylation status at the *BDNF* promoter 4 in BPD patients and controls.

#### 3.2.3.2. BDNF 81B

As well as at the promoter 4, testing markers rs2030324, rs988748, rs6265 and rs7124442 for modulatory effects on the methylation status at the *BDNF* promoter 4 in BPD patients and controls, no significant modulation was identified.
4. Discussion

Genetic and environmental contributions to BPD are often examined. Yet, only little is known about the role of the BDNF gene and the BDNF methylation levels in BPD patients. Moreover, until now no data exists, which investigates the effect of I-DBT on methylation levels in BPD.

The present study provides data to fill in existing gaps in this field. The genetic part focused on genotyping BPD patients and healthy controls at 6 positions on the BDNF gene (BDNF Microsatellite, rs2030324, rs988748, BDNF-LCPR, rs6265, rs7124442). The epigenetic part on the other hand, compared the methylation levels between BPD patients and healthy controls as well as methylation levels in BPD patients before and after they completed I-DBT. Methylation levels were assessed at two markers on the BDNF gene (BDNF promoter 4, BDNF 81B).

The results of the genetic part suggest that BPD patients differ significantly from healthy controls. In detail, single-marker analysis provided evidence for a difference at rs988748. The allele G seems to be protective for BPD, while allele C seems to favour BPD. Moreover, genotype G / G seems to be protective for BPD, while allele C / C seems to favour BPD. However, the C / G genotype is not significant. Thus, it seems that the combination of allele C and G neutralizes the protective and contributing effect on the development of BPD.

On the contrary, Dmitrzak-Weglzarz et al. (2008) did not find a different distribution among bipolar affective disorder and healthy controls at rs988748. However, they found the haplotype GC (rs988748/rs203024) of the BDNF gene to be significantly more frequent in patients with bipolar affective disorder than in controls (p=0.006) (Dmitrzak-Weglzarz et al., 2008). Hence, the haplotype GC (rs988748/rs203024) favours bipolar affective disorder. In contrast the same haplotype is protective for BPD. This is surprising, since bipolar disorder I often co-occurs with BPD (Grant, et al., 2008).

Schumacher et al. (2005) compared allele and genotype frequencies of rs988748 between patients with major depressive disorder (MDD), bipolar affective disorder, schizophrenia and control subjects. Neither allele nor genotype frequencies of rs988748 were significantly distributed in the four groups (Schumacher et al., 2005).

As a whole, this result provides new information, namely that patients with a mental disorder such as BPD differ significantly from healthy controls at rs988748. Until now, single-marker analysis did not provide evidence for association between rs988748 and different mental disorders. Having said that, it is important to know, that the Hardy-Weinberg-equilibrium is just on the limit (0.051) for that SNP. Since we did not control for ethnicity, thus, it might be possible, that controlling for ethnicity would show a non-significant result.

Besides the significant difference at rs988748 between BPD patients and controls, BDNF microsatellite 104 and 106 were also found to be significantly different distributed amongst these groups. While the occurrence of allele 104 seems to be protective for BPD, the occurrence of allele 106 seems to favour BDP. In addition, analyses of microsatellite-based genotypic associations showed that the BDNF microsatellite is significantly associated with BPD. Several genotypic combinations (94 / 100, 98 / 102, 100 / 102) were found to be significant, although the single alleles of the combinations were not significant themselves. These combinations show that not only single alleles, but also a combination can make the significant difference between cases and controls.
Taken together, this data clearly suggests that certain markers on the BDNF gene, namely the newly discovered BDNF microsatellite and SNP rs988748, contribute to the genetic risk of BPD.

The haplotype analysis compared two-marker combinations of BPD patients and controls. 29 haplotypes were found to differ significantly among these two groups. It is for the first time that such a relation could be shown.

Surprisingly, unequal values for BDP and controls were found, while testing the 6 markers for LD. Whereas no markers are linked in the healthy control group, the markers rs988748 and rs7124442 and the markers rs6265 and rs7124442 are strongly linked (Global D'value: 1) in BPD patients. An explanation for this abnormality might be the sample size of only 112 patients and 114 controls as well as the program Unphased, which is not the most appropriate to calculate LDs.

Results of the epigenetic part suggest that BPD patients have significant higher methylation levels on average than controls. This result is consistent with the finding of Carrard, Salzmann, Malafosse and Karege (2011), who also found a higher methylation levels in schizophrenia and bipolar disorder (Carrard, Salzmann, Malafosse, & Karege, 2011). This hypermethylation may result in a negative regulation of the BDNF expression (Keller, et al., 2010) or another gene expression (Jaenisch & Bird, 2003). BDNF promoter 4 is a promoter region extending over the initiation site for gene transcription. Thus, increase in methylation could have an impact on the gene transcription by impeding the interaction of the gene and transcription factors or RNA polymerase II as Carrard et al. (2011) have suggested (Carrard et al., 2011).

Furthermore, BPD patients before having started I-DBT have significant lower mean levels of methylation than after having accomplished therapy. This tendency is against expectations and thus requires explanations. Unfortunately no study addresses the question if and how therapy, DBT respectively, affects methylation levels in BPD. It is therefore difficult to interpret this result, when no comparison can be made with pre-existing data.

Nevertheless, it is strength of the present study that it focuses on the effect of psychotherapy on the score of DNA methylation in BPD.

A further strength presents the fact, that DNA methylation is examined in patients who also often suffer from other mental disorders. If high methylation levels mean vulnerability for mental disorders in general, high methylation could explain comorbidity, which is high in BPD and other mental disorder (Grant, et al., 2008). This fits well with the thoughts of Sant'Anna et al. (2007) which suggest the possibility of decreased BDNF levels in playing a crucial role for increased comorbidity (Kauer-Sant'Anna, et al., 2007). However, further studies are needed to confirm this assumption.

There are also some limitations, concerning different aspects of the study. First of all, the variable antidepressant intake was not controlled for. This might have been necessary nonetheless, since antidepressants intake alters methylation levels (Duman, 2004; Duman & Monteggia, 2006) and BDNF mRNA (Nibuya, Morinobu, & Duman, 1995). For that reason, further studies, which control for antidepressants are required.

Second, the included samples were heterogeneous. It is necessary to control for factors like ethnicity, comorbidity, gender, age, and so on.

Third, for practical and economic reasons, DBT was only applied to BPD patients. As a result, the data on the effect of DBT is limited to BPD only. To get a broader knowledge of the effect of DBT, study
designs are needed which investigate the effect of DBT in healthy populations and replicate the findings of this study. Only then, a clear statement about the effect of DBT on methylation levels can be made.

Fourth, only peripheral tissue was used. The relationship between methylation of blood and brain DNA is not well known. Thus, results are needed to be replicated in brain tissue, a technical problem that is almost impossible to overcome.

Also, sequencing bisulfite-modified DNA relies on the conversion of every single unmethylated cytosine to uracil. If conversion is incomplete, the subsequent analysis will incorrectly interpret the unconverted unmethylated cytosines as methylated cytosines, resulting in false positive results for methylation. Only cytosines in single-stranded DNA are susceptible to attack by bisulfite, therefore denaturation of the DNA undergoing analysis is critical (Fraga & Esteller, 2002).

Sixth, instead of sub-dividing BDNF-LCPR in 3 regions as Okada et al. (2006) did, we considered the BDNF-LCPR a a single region. For that reason, it is not possible for us to determine where exactly on the BDNF-LCPR the repetition occurs. In addition to this, we did not sequence the BDNF-LCPR in each patient (Okada, et al., 2006).

As Licino et al. (2010) suggested, extensive resequencing of key candidate genes can lead to the discovery of substantial numbers of variants, which might be associated with a specific psychiatric disorder and treatment response (Licino et al., 2010). The study in hand presents an attempt to find a relevant gene and its belonging variants. Still, many questions remain open and further research in this field is required. It has to be shown what role BDNF-gene x interaction plays in mental disorders, such as BPD. Haplotypes beyond one gene may suggest a predisposing role in regard to BPD. A first contribution was already done by Tadic et al. (2009). They found an association of the serotonin transporter gene (HTR1B A-161) and BDNF gene (196A) in BPD (Tadic et al., 2009).

Moreover, whether methylation levels are modulated by length polymorphisms (BDNF promoter 4, BDNF microsatellite) was not tested and has to be addressed in future research.

Besides this, prospective projects should also include BDNF proteins to correctly assess the correlation between methylation levels and BDNF protein levels.

In conclusion, the present data suggests that BDNF and also its methylation status may be a susceptible gene contributing to BPD pathophysiology. BPD patients and healthy controls differ from each other genetically (BDNF microsatellite, rs988748) and epigenetically (BDNF promoter 4, BDNF 81B). In addition to this, it seems that I-DBT can change methylation levels in BPD patients. Taking into account the limitations of the present study, this data needs to be replicated and further analyses have to be done before reaching a final conclusion.
5. References


Genetic & epigenetic differences between borderline personality disorder patients & healthy controls


Licinio, J., Dong, C., & Wong, M. L. (2009). Novel sequence variations in the brain-derived neurotrophic factor gene and association with major depression and antidepressant


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