Decreased serum levels of brain-derived neurotrophic factor in adults with attention-deficit hyperactivity disorder

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Abstract

It has been hypothesized that brain-derived neurotrophic factor (BDNF) is involved in the pathogenesis of attention-deficit hyperactivity disorder (ADHD), although experimental data regarding the contribution of BDNF gene polymorphisms to this psychiatric disorder are controversial. Recently, changes in BDNF serum levels have been reported in children with ADHD, but there are no studies about the possible role of this neurotrophin in adults. A total of 54 Caucasian ADHD adults, including the predominantly inattentive and combined types (aged 33.43 ± 8.99 yr) and 59 Caucasian unrelated healthy controls (aged 35.52 ± 9.37 yr) were included in a study to evaluate BDNF levels in serum. Medical, neurological and psychiatric co-morbidities were excluded. Clinical data concerning ADHD diagnosis and blood samples for patients and controls were collected. BDNF serum levels were significantly lower in adults with ADHD compared to healthy controls (p < 0.0001). Although the combined type of ADHD subgroup displayed lower BDNF serum levels than the inattentive type, the differences did not reach statistical significance. No significant correlations were found between serum BDNF levels and scores on the Conners’ Adult ADHD Rating Subscales. These results suggest a role for BDNF in ADHD, at least in those patients whose disorder persists throughout life. Low BDNF levels may contribute to the neurodevelopmental deficits of ADHD and to the persistence of the disorder into adulthood. BDNF differences between ADHD subtypes should be further studied.

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Introduction

Attention-deficit hyperactivity disorder (ADHD) is a psychiatric condition that is defined by the core symptoms of inattention, hyperactivity and impulsivity and that begins in childhood, before the age of 7 yr, according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Revised (DSM-IV-TR). Symptom intensity, especially hyperactivity, has been shown to decrease over time (Hart et al., 1995; Mick et al., 2004); however, for many patients the disorder persists into adulthood, although in some of them only some impairing symptoms remain (Rasmussen and Gillberg, 2000). The estimated prevalence of ADHD in adults ranges from 2.9% (Faraone and Biederman, 2005) to 4.4% (Kessler et al., 2006).

Although the underlying pathogenesis of ADHD is still not well established, it is already accepted that it has a multi-factorial neurodevelopmental origin with a strong genetic component, with an estimated heritability of approximately 60% (Biederman and Faraone, 2005). Environmental risk factors also play a role in ADHD, especially if they are present in the pre- and early post-natal periods during the development of the brain (Galéra et al., 2011; Sagiv et al., 2012). From a neurobiological point of view, different lines of evidence suggest the involvement of the dopaminergic and serotoninergic systems (Solanto, 2002; Ribasés et al., 2009; Landaas et al., 2010; Nijmeijer et al., 2010) in the pathogenesis of ADHD. Experimental studies have shown a strong relationship between these monoamnergic systems and a member of the neurotrophin family, brain-derived neurotrophic factor (BDNF; Küppers and Beyer, 2001; Dluzen et al., 2002; Goggi et al., 2003), which is widely expressed in the mammalian brain (Leibrock et al., 1992). BDNF has an important role in the development of the dopaminergic system (Yurek et al., 1996; Küppers and
In the mature central nervous system, BDNF plays an essential role in neuronal survival, modulating activity-dependent neuronal plasticity and synaptic proliferation and facilitating learning and the ability to adapt to environmental requirements (Aicardi et al., 2004; Chapleau et al., 2009; Fritsch et al., 2010). The most frequently studied single nucleotide polymorphism (SNP) of the BDNF gene (Petryshen et al., 2010), the Val<met SNP, is associated with changes in intracellular trafficking and secretion of protein (Chen et al., 2004), which have functional consequences, for example, in hippocampal-dependent learning (Linnarsson et al., 1997), aggressiveness (Ito et al., 2011) and locomotor hyperactivity (Kernie et al., 2000; Rios et al., 2001).

Genetic studies in patients with ADHD have attempted to assess the role of BDNF gene polymorphisms in the disease, although the results are controversial. Whereas some authors have reported a positive association between the Val<met BDNF polymorphism and ADHD (Kent et al., 2005; Lanktree et al., 2008; Bergman et al., 2011), others have failed to find such an association (Lee et al., 2007; Schimmelmann et al., 2007). A meta-analysis using a large sample of adults with ADHD did not find evidence for the involvement of the Val/Met polymorphism in this psychiatric disorder (Sánchez-Mora et al., 2010). Other polymorphisms of the BDNF gene, such as C270T, have also been associated with ADHD (Xu et al., 2007; Aureli et al., 2010), although their functional significance still has not been elucidated. On the other hand, experimental studies have shown that early life experiences can induce long-lasting changes in BDNF gene expression. The word ‘epigenetic’ is used to refer to a heritable phenotype caused by mechanisms other than changes in the DNA sequence (Nestler 2009). Epigenetics refers to functionally relevant modifications, such as DNA methylation or histone acetylation, in the regulation of gene expression that do not modify the underlying DNA sequence (Fuchikami et al., 2010). Alterations in the epigenetic regulation of BDNF genes mediate the effects of environmental risk factors, resulting in enduring changes in the expression and function of the BDNF protein (for a review, see Roth and Sweatt, 2011; Boulle et al., 2012).

Previous data suggest that there is motivation for assessing the role of the serum BDNF in ADHD because this neurotrophin could be a biological marker for this disorder. Studies of BDNF perfusion showed that this neurotrophin is able to cross the blood–brain barrier in both directions (Pan et al., 1998). An animal model of electroconvulsive therapy reported that, after some electroconvulsive shock sessions, the temporal expression profile of BDNF in the brain was associated with the rise of this neurotrophin in serum, also indicating that BDNF crosses the blood–brain barrier (Sartorius et al., 2009). The significant positive correlation between BDNF in serum and in cortical brain regions (Karege et al., 2002) as well as between blood and hippocampus BDNF (Klein et al., 2011), reported in young and mature rats, further underlies the potential for peripheral measures of BDNF as a biomarker. One study using plasma measures has revealed abnormal BDNF expression in untreated children with ADHD, finding a significant positive correlation between BDNF and the severity of inattentive symptoms (Shim et al., 2008). To our knowledge, however, there are no data regarding serum BDNF expression in adults with ADHD. In the present study, we aimed to assess possible changes in serum BDNF levels in adults with ADHD compared to healthy controls and to study possible differences between ADHD subtypes. We hypothesize that a decrease of BDNF expression may play a role in the pathogenesis of ADHD and that this decrease persists into adulthood.

Materials and method

Subjects

The clinical sample comprised 54 Caucasian adults with ADHD who were recruited from the Program for Adults with ADHD in the Department of Psychiatry of the Hospital Universitari Vall d’Hebron between 2008 and 2010. All of the patients met the diagnostic criteria of ADHD, according to the DSM-IV-TR criteria. Exclusion criteria for both groups included the following: (1) lifetime and current history of mood, psychotic, anxiety, substance abuse and DSM-IV Axis II disorders; (2) having history or any current condition or illness including neurological, metabolic, cardiac, liver, kidney or respiratory disease; (3) any other medical illness that can interfere with the expression of BDNF, such as overweight or obesity; (4) allergy; (5) taking chronic medication of any kind; (6) having an IQ <70; (7) pregnancy or breastfeeding. The control sample comprised 59 Caucasian unrelated healthy adults recruited from the Blood and Tissue Bank at Hospital Universitari Vall d’Hebron, who were matched with the patients for sex and age and in whom DSM-IV ADHD diagnosis was excluded.

The study was approved by the Ethics Committee of Vall d’Hebron University Hospital and written informed consent was obtained from all of the adult subjects, patients and controls.

Clinical assessment

The diagnosis of ADHD was evaluated with the Conners’ Adult ADHD Diagnostic Interview for DSM-IV-TR (Part II; Epstein et al., 1999) and co-morbidity was evaluated with the Structured Clinical Interview for DSM-IV Axis I.
and Axis II Disorders (SCID-I and SCID-II). The severity of ADHD symptoms was evaluated with the long version of the Conners’ Adult ADHD Rating (CAARS-S; Conners et al., 1999), which is a self-report that includes four-empirically derived subscales: (a) inattention/memory problems; (b) impulsivity/emotional lability; (c) hyperactivity/restlessness; (d) problems with self-concept. The CAARS-S also includes ADHD symptom measures that help in the assessment of inattentive symptoms, hyperactive–impulsive symptoms and total ADHD symptoms. The Wender Utah Rating Scale (Ward et al., 1993) was also used to assess retrospective symptomatology. To exclude current symptoms of depression and anxiety, often present in ADHD, patients fulfilled the Hamilton Rating Scale for Depression (HAM-D; Hamilton, 1960) and the State and Trait Anxiety Inventory (STAI; Spielberger et al., 1983). IQ was estimated with the Vocabulary and Block Design subtest of the Wechsler Adult Intelligence Scale – Third Edition. All of the patients were off medication for at least 4 wk at the time of testing.

**Blood sample collection**

For serum sampling, 8 ml blood was collected from the antecubital vein in anticoagulant-free tubes and was kept at 4 °C for 2 h. All of the samples were collected between 10:00 hours and 12:00 hours, to avoid circadian variation. The samples were kept in the refrigerator for 2 h and then centrifuged at 2465 g x 10 min at 4 °C. Serum was carefully collected and kept at −80 °C until the assay to determine the BDNF level.

**Measurement of serum BDNF**

The level of human BDNF in serum samples was measured using the Aushon SearchLight Multiplex Array (Aushon BioSystems, USA), a sandwich enzyme-linked immunosorbent system for quantitative protein measurement. In this assay, samples and standards are added to the wells and proteins within the samples bind the captured antibodies. The integrated values for known standards are used to generate standard curves. We analysed each sample twice and used the mean of the two BDNF measurements. All of the mean intra-assay coefficients of variation were <20%.

**Statistical analysis**

To analyse the differences between the means of the ADHD and the control subjects, a t test was performed. To compare the BDNF levels between the combined and inattentive subjects, a t test was performed. Zero-order correlations were computed to analyse the associations between the BDNF level and the CAARS subscales. The effect of age and sex on the BDNF level was analysed with a zero-order correlation and a t test, respectively.

The error α assumed was 5%. SPSS version 18 was used to process the data.

**Results**

**Demographic characteristics and evaluation of clinical symptoms**

The group of patients included 54 adults with ADHD, 37 males and 17 females, with ages ranging from 18 to 55 yr (mean 33.43 ± 8.99). The group of healthy controls included 38 males and 22 females, with ages ranging from 18 to 55 yr (mean 34.07 ± 9.40). Of these, 15 of the adult patients fell into the inattentive subtype and 38 fell into the combined subtype, according to the DSM-IV. The severity of the ADHD symptoms across the clinically significant domain was also assessed using the CAARS-S. The mean and s.d. for the different CAARS subscales are reported in Table 1.

Table 2 shows the mean and s.d. of the CAARS subscales for both the combined and the inattentive subtypes. T test comparisons revealed significant differences between the inattentive and combined subtypes for hyperactive/restlessness, DSM-IV hyperactive–impulsive, DSM-IV ADHD symptoms total and problems with self-concept. Nevertheless, after using Bonferroni’s correction for multiple comparisons, only the differences between the inattentive and combined subtypes for the DSM-IV hyperactive–impulsive subscale remained statistically significant.

**Comparison of serum BDNF between adults with ADHD and controls**

The mean and s.d. serum BDNF level was 52.13 ± 20.76 ng/ml for adult patients and 69.77 ± 23.20 ng/ml for normal controls. T test comparisons show that the BDNF serum level was significantly lower in adults with ADHD compared to healthy controls (t = 4.243; p < 0.0001).

The study of the correlation between serum BDNF and age revealed no effect of age (t = 0.029; p = 0.758) on the BDNF level in whole samples, including patients and controls. T test comparisons revealed no sex differences in BDNF serum levels for patients (t = 0.130; p = 0.897) or healthy controls (t = 0.028; p = 0.978).

**Comparison of serum BDNF between different ADHD subtypes**

When using the DSM-IV criteria to classify ADHD patients into the inattentive or combined subtypes, t test comparisons showed no significant differences in the BDNF serum level between both subtypes (t = 1.032; p = 0.307). Nevertheless, BDNF levels in the combined subtype were lower than in the inattentive subtype. The mean serum level in the combined subgroup was 50.25 ± 17.49 ng/ml; whereas in the inattentive subgroup, the mean serum level was 56.84 ± 28.10 ng/ml. Figure 1 depicts the mean serum BDNF levels for the group of healthy controls and for the subgroups of combined and inattentive ADHD patients.
No significant correlations were found between serum BDNF levels and scores on the CAARS subscales. The highest correlation was 0.185 for the DSM-IV ADHD symptom total subscale.

Discussion

To our knowledge, this is the first study on serum levels of BDNF in adult patients with ADHD. The results show a significant decrease in serum BDNF in adults with ADHD compared with normal controls. Importantly, co-morbidities, including personality disorders, current anxiety, depressive and substance use disorders were excluded. Additionally, all of the patients were off medication for at least 1 month before measuring the BDNF serum level. Therefore, the decrease in serum BDNF cannot be attributed to co-morbidities or to an effect of previous medication. In this regard, our results
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support the hypothesis that a deficit in central BDNF
could play a role in the pathophysiology of ADHD.

From this study, there could be different explanations
for the decrease in serum BDNF levels found in adults
with ADHD. First, the decrease in serum BDNF could be related to the BDNF gene polymorphisms that are in-
volved in ADHD. Although some authors have failed to
find an association between the Va**Met polymorphism and ADHD in children (Lee et al., 2007; Schimmelmann
et al., 2007) or in adults (Ribasé et al., 2008; Landaa
es et al., 2010), others have reported an association between
the Met variant (Lasky-Su et al., 2007; Bergman et al.,
2011) or the Val allele (Kent et al., 2005; Lanktree et al.,
2008) of the BDNF gene and ADHD. In the general
population, the Val/Val genotype was linked to lower
serum BDNF levels than the Val/Met genotype (Lang
et al., 2009). Additionally, the C270T polymorphism of
the BDNF gene has also been associated with ADHD
(Xu et al., 2007; Aureli et al., 2010); nevertheless, its
functional significance has not been disclosed.

Second, epigenetic factors could also be involved in
the decrease in BDNF expression in adults with ADHD. Epigenetics include the key mechanisms by which ad-
verse foetal or early childhood environments could in-
duce changes in BDNF gene expression and subsequent
BDNF protein availability, resulting in altered beha-
vioural outcomes (for a review, see Roth and Sweat,
2011; Boulle et al., 2012). For example, in mice, early
maternal deprivation leads to an increase in corticoste-
one and a decrease in BDNF expression, which is associ-
ated with the inhibition of hippocampal cell proliferation
(Kikusui et al., 2009). In humans, prenatal maternal
smoking leads to an increase in DNA methylation in the
promoter region of the BDNF gene, which, in turn,
induces the long-term down-regulation of BDNF ex-
pression (Toledo-Rodriguez et al., 2010). Additionally,
stressful events experienced during pregnancy predicted
ADHD traits in a cohort of 2900 pregnant women (Ronald
et al., 2011). In this regard, diverse polymorphisms of the
BDNF gene have been associated with ADHD but only
when controlling for socio-economic status (Lasky-Su
et al., 2007), suggesting the involvement of epigenetic
mechanisms at the interface between BDNF polymor-
phisms and ADHD symptoms. Together, these results sug-
gest that exposure to adverse environmental factors,
especially when they are present during the foetal or
early life stages, might be responsible for the neurode-
velopmental deficits underlying ADHD through changes in
BDNF availability.

We cannot rule out the possibility that the decrease of
BDNF in serum found in our study may be secondary to
a dysfunction of the diverse neural systems involved
in ADHD. For example, in these patients relative to controls,
the frontostriatal circuitry is hypofunctional in tasks
involving executive functions and the mesocorticollimbic
regions are hypoaffective in a reward-processing task. At
the same time, ADHD patients also show enhanced activa-
tion in some other brain regions, which reflects diffi-
culties with deactivation of the default mode network and
interfere with the task in hand (Sonuga-Barke and
Castellanos, 2007; for a review, see Cortese et al., 2012). It
is well established that BDNF is released in the brain
in response to neural activity (Thoenen, 1995). Since,
BDNF can cross the blood–brain barrier (Pan et al., 1998;
Sartorius et al., 2009), and a positive relationship has been
found between brain and blood BDNF protein levels in
rodents (Karege et al., 2002; Sartorius et al., 2009; Klein
et al., 2011), changes in serum levels of BDNF would
result from the influence of both positive and negative
activity, in multiple brain regions.

One of the most frequent confounding factors when
studying serum BDNF levels is depressive symptoms.
Depression has been consistently associated with a de-
crease of BDNF in serum (Sen et al., 2008), which
ormalizes with antidepressant treatment (Molendijk et al.,
2011). This would not be of concern in our study since we
included only patients without past or current depressive
disorder. All of the patients in the study had scores
<5 on the HAMD, showing that depressive symptoms were
not present. On the other hand, low BDNF levels have
been reported in female patients with anxiety disorders
(Molendijk et al., 2012). Past and current co-morbid an-
xiety diseases were excluded from our study and, when
explored with State-STAI, all the patients had scores <5.
Additionally, no sex differences in serum BDNF levels
were found in ADHD patients. Together, these data fur-
ther suggest that low serum BDNF levels are a charac-
teristic of ADHD patients.

The decreased serum BDNF levels in adults with
ADHD appear to contradict the results of Shim et al.
that the Val
2012), although the nature of these sleep alterations is still
disturbances both in children (Lecendreux and Cortese,
and memory difficulties that characterize ADHD (Rohlf
et al., 2004; Chapleau et al., 2009; Fritsch et al., 2010) and
samples of ADHD patients.
Given the role of BDNF in synaptic plasticity (Aicardi
et al., 2004; Chapleau et al., 2009; Fritsch et al., 2010) and
learning (Linnarsson et al., 1997), low BDNF in serum
can be associated with certain clinical characteristics in
patients with ADHD. For example, some of the cognitive
and memory difficulties that characterize ADHD (Rohlf
et al., 2012) may be explained, at least in part, by a de-
creased functionality of the BDNF system. There is also
increasing evidence that ADHD is associated with sleep
disturbances both in children (Lecendreux and Cortese,
2007; Konofal et al., 2010) and in adults (Fargason et al.,
2012), although the nature of these sleep alterations is still
not clear. Recently, evidence has been found indicating that
the Val<sup>66</sup>Met polymorphism of the BDNF gene
has been involved in the modulation of sleep intensity
(Bachmann et al., 2012) in healthy humans. In this con-
text, the decrease of serum BDNF reported in the present
study suggests that BDNF may contribute to sleep dis-
turbances in adults with ADHD. Further studies ad-
ressing the possible relationship between BDNF, sleep
disturbances and cognitive deficits in ADHD are needed.
We acknowledge that our study has several limita-
tions. First, the small number of patients included in each
subgroup of ADHD adults reduces the validity of com-
parisons between the inattentive and combined subtypes.
Second, external factors such as exercise (Ferreira et al.,
2011; Heyman et al., 2012) or diet (Godar et al., 2011)
could influence BDNF expression in the serum, hindering
the use of serum BDNF as a biomarker of disease.
Nevertheless, we believe that these two sources of vari-
ability in serum BDNF levels could be balanced when
comparing the samples of patients and controls.
In conclusion, our results show a decrease in serum
BDNF levels in adults with ADHD, which suggests a role
for BDNF, at least in those patients whose disorder per-
sists throughout life. Although a trend towards a lower
BDNF level was found in the combined subtype com-
pared to the inattentive subtype, larger studies will be
needed to further explore these differences. Additionally,
lontudinal studies with larger samples of patients that
assess the evolution of serum BDNF throughout the dif-
ferent post-natal stages could be warranted to clarify the
involvement of BDNF in diagnostic continuity and clin-
ical characteristics of patients with ADHD. Furthermore,
our data suggest that, in addition to possible genetic
polymorphisms, epigenetic mechanisms mediating the
effects of environmental risk factors could also play a role
in the decrease of serum BDNF levels in these patients.

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Statement of Interest
None.

References
Aicardi G, Argilli E, Cappello S, Santi S, Riccio M, Thoenen H,
Canossa M (2004) Induction of long-term potentiation and
depression is reflected by corresponding changes in secretion
of endogenous brain-derived neurotrophic factor. Proc Natl
Acad Sci USA 101:15788–15792.
Aureli A, Del Beato T, Sebastiani P, Marimpietri A, Melillo CV,
disorder and intellectual disability, a study of association with
brain-derived neurotrophic factor gene polymorphisms. Int J
Bachmann V, Klein C, Bodenmann S, Schäfer N, Berger W,
Brugger P, Landolt HP (2012) The BDNF Val<sup>66</sup>Met
polymorphism modulates sleep intensity: EEG frequency-
and state-specificity. Sleep 35:35–44.
relay zone in the hippocampal formation occurs in the human
brain during childhood, adolescence, and adulthood. Arch
Gen Psychiatry 51:477–484.


