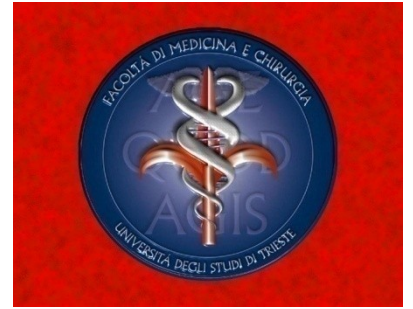


**Department of Medical,
Surgical and Health
Sciences**

University of Trieste



**PhD program in
LIAISON AND CONSULTATION PSYCHIATRY**

PhD Thesis

***BDNF as a biomarker in
psychiatry***

PhD Student:

dott. **Daide CARLINO**

Coordinator:

Chiar.mo Prof. **Maurizio DE VANNA**

Supervisor:

Chiar.mo Prof. **Enrico TONGIORGI**

Academic year 2010-2011 (XXIV cycle)

Names and full addresses of the committee

Coordinator:

Prof. Maurizio De Vanna
Dipartimento di Scienze Mediche, Chirurgiche e della Salute - Università degli studi di Trieste
Via Paolo De Ralli 5 – 34127 Trieste

Supervisor:

Prof. Enrico Tongiorgi
Dipartimento di Scienze della Vita - Università degli studi di Trieste
Via Giorgieri 5 – 34100 Trieste

President of the committee:

Prof. Maurizio De Vanna
Dipartimento di Scienze Mediche, Chirurgiche e della Salute - Università degli studi di Trieste
Via Paolo De Ralli 5 – 34127 Trieste

Committe members:

Prof. Paolo Santanastaso
Dipartimento di Neuroscienze – Università di Padova
Via N. Giustiniani 2 – 35100 Padova

Prof. Angela Favaro
Dipartimento di Neuroscienze – Università di Padova
Via N. Giustiniani 2 – 35100 Padova

Prof. Enrico Tongiorgi
Dipartimento di Scienze della Vita - Università degli studi di Trieste
Via Giorgieri 5 – 34100 Trieste

Prof. Eugenio Aguglia
Dipartimento di Biomedica Clinica e Molecolare – Università di Catania
Via Santa Sofia 78 – 95121 Catania

Prof. Giuseppe Maina
Dipartimento di Neuroscienze – Università di Torino
Via Cherasco 15 - 10126 Torino

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Abstract

Brain-derived neurotrophic factor (BDNF) is a key factor in learning and memory. Altered BDNF-signalling is thought to contribute to the pathogenesis of schizophrenia and major depression (SZ) especially in relation to cognitive deficits. However, analysis of serum BDNF as a potential biomarker in psychiatry has provided controversial data. We hypothesized that these confounding results might be due to a differential regulation of BDNF precursor pro-BDNF (32 KDa) and proteolytic products mature (mat-BDNF; 14 KDa), and truncated-BDNF (28 KDa).

Because of these discrepancies, we decided to perform a systematic review and a meta-analysis of studies measuring serum concentrations of BDNF to elucidate whether or not this neurotrophin is abnormally produced in patients with schizophrenia.

Additionally, we were interested in identifying factors that might contribute to the different findings in literature, as to improve the design of future investigations in this field.

In the second part of thesis, we investigated the serum abundance of these BDNF isoforms and its relationship with cognitive impairment in schizophrenia. Schizophrenia was diagnosed with PANSS test. Abbreviated cognitive assessment included tests for attention, perceptualmotor skills, processing speed and memory. Using an ELISA assay, we found a slight reduction in serum BDNF levels in SZ patients ($n = 40$) with respect to healthy controls (HC, $n = 40$; $p = 0.018$). Western-blot analysis revealed increased serum pro-BDNF and mat-BDNF and reduced truncated-BDNF ($p < 0.001$) in SZ with respect to HC. Patients with an increase in pro-BDNF ($n = 15/40$) or mat-BDNF ($n = 9/40$) higher than the HC mean ± 2

Standard Deviations (SD) also had >2SD reduction of truncated-BDNF (n = 27/40). Reduced truncated-BDNF correlated significantly with higher positive and lower negative PANNS scores and a worst performance in all cognitive assays but not with antipsychotic type. Measurement of serum truncated-BDNF abundance predicted for high cognitive deficits with sensitivity $\frac{1}{4}$ 67.5%, specificity $\frac{1}{4}$ 97.5%, Negative Predictive Value = 75% and Positive Predictive Value = 96.4%. These results suggest deficiency in pro-BDNF processing as a possible biological mechanism underlying schizophrenia with cognitive impairment. Finally, future perspectives on the use of proBDNF as a novel biomarker for psychiatric disorders will be discussed.

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Carlino D, De Vanna M. *Patologie complesse ed isolati genetici*. Italian Journal of Psychopathology vol.16, march 2010: 125.

De Vanna M, Zangrando A, Moret V, Terrana R, **Carlino D**. *Serum Brain-derived neurotrophic factor (BDNF): determinants and relationship with depressive and anxious symptoms in an isolated population*. Congresso internazionale INNOPSY 2011 - Innovazione in Psichiatria - Milano

De Vanna M, Zangrando A, Moret V, Terrana R, **Carlino D**. *Genome Wide Association Study Of Alcohol Abuse In Genetic Isolated Populations*. Congresso internazionale INNOPSY 2011 - Innovazione in Psichiatria - Milano

De Vanna M, Zangrando A, Moret V, Terrana R, **Carlino D**. *Role of shared genetic and environmental factors in symptoms of depression and anxiety*. Congresso internazionale INNOPSY 2011 - Innovazione in Psichiatria – Milano.

De Vanna M, Moret V, Terrana R, **Carlino D**. *Patologie stress correlate ed isolati genetici*. Italian Journal of Psychopathology vol.17, march 2011: 115.

Carlino D, Sheila Ulivi S, D'Adamo P, De Vanna M, Gasparini P. Genetico of panic disorder in an isolate population: the role of DPP10 gene. *Congresso Società Italiana di Genetica Umana – SIGU - 2011*

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Sheila Ulivi S, D'Adamo P, Gasparini P, **Carlino D**, De Vanna M. *The genetics of Suicide Behaviour*. European Human Genetics - Conference 2010.

Carlino D, De Vanna M. [Potential benefits and limitations of new technologies in the personalized treatment of depression]. Riv Psichiatr. 2011 May-Jun;46(3):155-71.

Carlino D, Leone E, Di Cola F, Baj G, Marin R, Dinelli G, Tongiorgi E, De Vanna M. Low serum truncated-BDNF isoform correlates with higher cognitive impairment in schizophrenia. *J Psychiatr Res.* 2011 Feb;45(2):273-9.

List of abbreviation

MDD: major depressive disorder

AD: Alzheimer's disease

ANOVA: Analysis of variance

BD: bipolar disorder

BDNF: brain-derived neurotrophic factor

CA1: *Cornus ammoni* 1

CA3: *Cornus ammoni* 3

CNS: central nervous system

CPT: Continuous Performance Test

CREB: cAMP response element-binding

DG: dentate gyrus

DSM-IV: Diagnostic and Statistical Manual of Mental Disorders

ELISA: Enzyme-Linked Immunosorbent Assay

mRNA: messenger Ribonucleic Acid

NGF: nerve growth factor

NT: neurotrophin

NTR: neurotrophin receptor

p75NTR: p75 Neurotrophin Receptor

PNS: Periferal Nervous System

1. Introduction

In the last three decades, a growing body of researches on cognition in neurodegenerative disorders, such as schizophrenia and Alzheimer's disease (AD), have been conducted. Several lines of evidence suggested that cognitive impairment significantly affects quality of life and global functioning in these clinical pictures, potentially more than other behavioral or positive symptoms (i.e. hallucinations or delusions) (Nuechterlein et al., 2011). One attractive candidate for modulating synaptic plasticity in cognitive processes as learning and memory is the Brain Derived Neurotrophic Factor (BDNF). The BDNF is a member of the neurotrophins' family, which includes the Nerve Growth Factor (NGF), the Neurotrophin-3 (NT-3) and the NT-4/5.

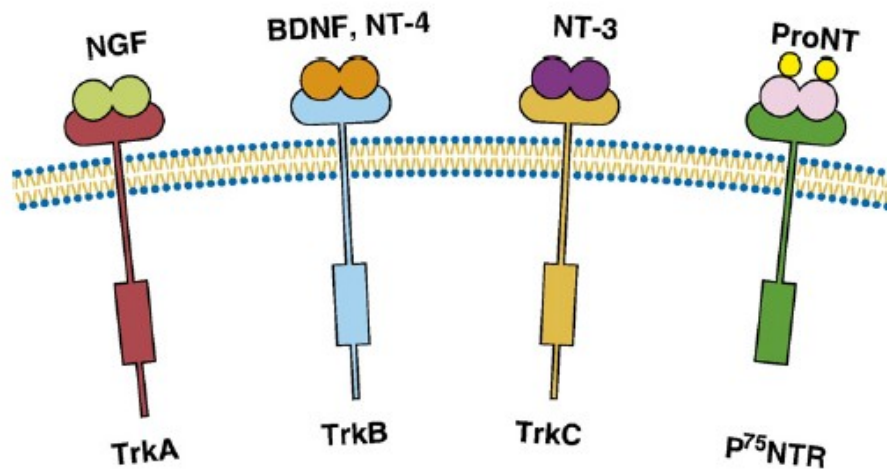


Figure. 1 Neurotrophins and their receptors.

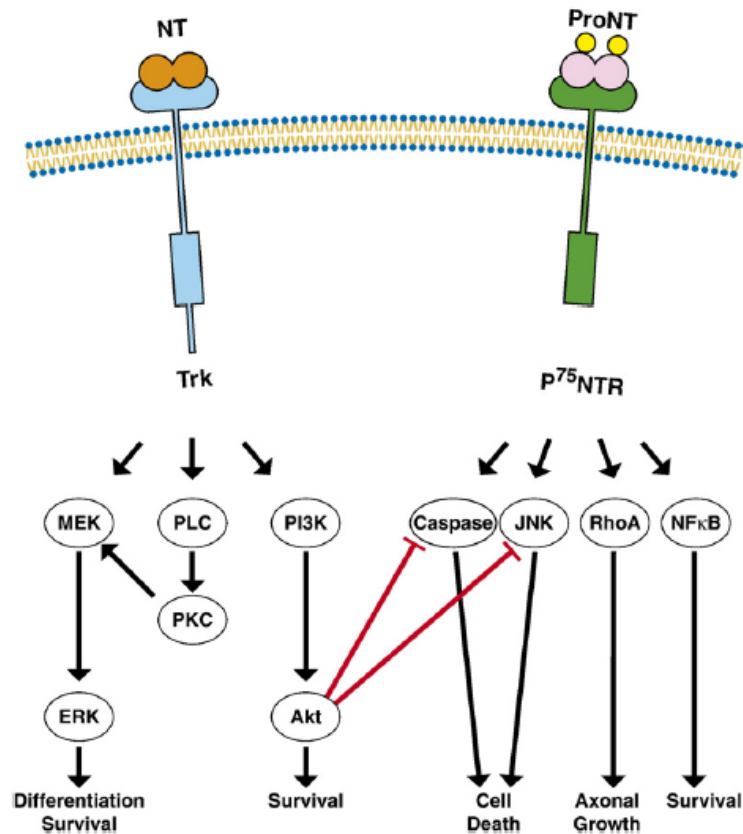


Figure 2. Transductional mechanism of action of neurotrophins and their precursors.

This neurotrophin is highly expressed in the central nervous system (CNS) and modulates several forms of synaptic plasticity (Schinder et al., 2000), especially in the hippocampus and cortex; additionally, it is involved in dopaminergic (Guillin et al., 2001), glutamatergic (Carvalho et al., 2008) and serotonergic (Mossner et al., 2000) neurotransmitter systems. In particular, BDNF *Val66Met* allele polymorphisms suggested that the BDNF is implicated in human memory. *Val66Met* mutation results in abnormal BDNF packaging into secretory granules, leading to decreased activity-dependent BDNF release (Egan et al., 2003). This alteration is associated to impaired episodic memory and executive performance (Miyajima et al., 2008; Schofield et al., 2009), reduced hippocampal activation (Egan et al., 2003; Hariri et

al., 2003; Dempster et al., 2005), and increased risk of AD (Tsai et al., 2004; 2006). Ho et al. (2006) showed that both healthy and schizophrenic Met allele carriers had poorer verbal memory performance than their Val/Val subjects, whereas visuo-spatial impairment was specifically found in patients with schizophrenia, but not in healthy volunteers. There were significant genotype effects on gray matter volumes within brain regions supporting memory and visuo-spatial functioning, with Met-allele-carriers having smaller parietal, temporal and occipital lobar gray matter. However, negative findings have been reported on a recent meta-analysis investigating the genetic association between BDNF Val66Met polymorphism and cognition (Mandelman et al., 2011). Decreased serum concentration of BDNF has been consistently described in AD patients (Einat et al., 2003; Laske et al., 2007; Lee et al., 2009; Forlenza et al., 2010) and age-related cognitive decline reflecting a decrease in BDNF in subregions of the brain (the frontal cortex, the hippocampus, the parietal cortex, the temporal cortex and the entorhinal cortex) that are primarily affected by age-related diseases (Laing et al., 2011). However, these studies on serum BDNF levels required further deepening as serum BDNF levels were significantly lower in AD patients than those in matched patients with vascular dementia and controls, but did not correlate with age or scores in MMSE or Functional Assessment Staging (FAST) (Yasutake et al., 2006). Nevertheless, in Laske et al. (2006) serum BDNF levels were not predictive of progression to AD or cognitive deterioration in MCI, even if the presence of the Met allele was a significant predictor of cognitive deterioration in these patients. In opposition to these findings, other investigators also reported on increased BDNF levels in patients with MCI and early AD (Angelucci et al., 2010). Also, recent clinical trials demonstrated that lithium and a cholinesterase inhibitor (donepezil) were able to increase serum BDNF levels

in patients with early AD (Leyhe et al., 2009). Increments in BDNF levels may suggest that to these drugs have some neurobiological effects in AD and MCI patients (Diniz et al., 2009; Forlenza et al., 2010). Niitsu et al. (2011) found no significant differences in serum BDNF levels between normal controls and patients with schizophrenia, although serum BDNF levels were associated to verbal working memory impairment and negative symptoms in patients with schizophrenia. Taking as model previous animal studies investigating learning-induced neuroplasticity (Buonomano et al., 1998), Vinogradov et al. (2009) showed that serum BDNF levels were significantly increased among clinically stable, chronically ill schizophrenia patients involved in neuroplasticity-based cognitive training. Here, we aimed to systematically review the literature 1) to explore the role of proBDNF in animal models of cognitive impairment and 2) to elucidate whether abnormalities of the proBDNF/mBDNF ratio may contribute to the pathophysiology of neuropsychiatric disorders.

2. BDNF biosynthesis

BDNF is initially synthesized as a precursor protein (prepro-BDNF) in the endoplasmic reticulum. Mammalian pro-BDNF precursor is also processed to truncated-BDNF 28 kDa (proBDNF) or mature 14 kDa BDNF (mBDNF) following two different proteolytic cleavages.

mBDNF is synthesized in the trans-Golgi by furin (Mowla et al., 2001), or extracellularly, by plasmin or matrixmetalloprotease-7 (Lee et al., 2001). proBDNF is generated by a specific Ca^{2+} -dependent serine proteinase known as Membrane-bound transcription factor site-1 protease (MBTFS-1), also known as Subtilisin/kexin-isozyme 1 (SKI-1) (Seidah et al., 1999). proBDNF is not further processed into the

mature 14 kDa BDNF form and it represents a true final proteolytic product whose function is still unclear. It has long been thought that only secreted mBDNF was biologically active, whereas pro-BDNF was an inactive precursor localized intracellularly. Recent studies demonstrated that mBDNF and pro-BDNF elicited opposite biological effects (Teng et al., 2005; Woo et al., 2005). Specifically, mBDNF bound Trk-B receptor promoting dendritic spines formation and proBDNF bound p75 receptor triggering apoptotic processes.

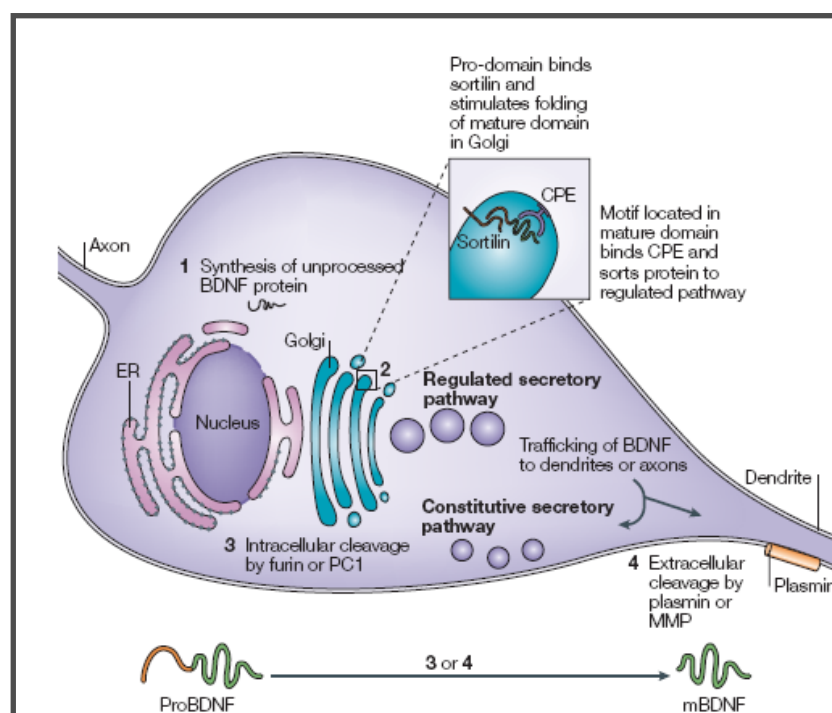


Figure 3. Synthesis and sorting of BDNF.

More recently, Koshimizu et al. (2009) found that the overexpression of pro-BDNF elicited the apoptosis of cultured cerebellar granule neurons and caused a dramatic reduction in the number of cholinergic fibers of basal forebrain neurons and hippocampal dendritic spines, without affecting the survival of these neurons. Conversely, blockade of p75 receptor prevented spine number reduction. These

evidences lead researchers to the conclusion that an incorrect balancing of the different isoforms may result in some neuropathological effects. It has been observed that BDNF and the tissue plasminogen activator (tPA, a serine protease) both were implicated in late-phase long-term potentiation (L-LTP) and long-term memory. *In vitro* conversion of proBDNF to mBDNF by plasmin *via* the tPA-dependent activation of plasminogen and mBDNF mediated L-LTP in tPA and plasminogen knock-out mice suggested the existence of a mechanistic link between tPA and BDNF (Pang et al., 2004).

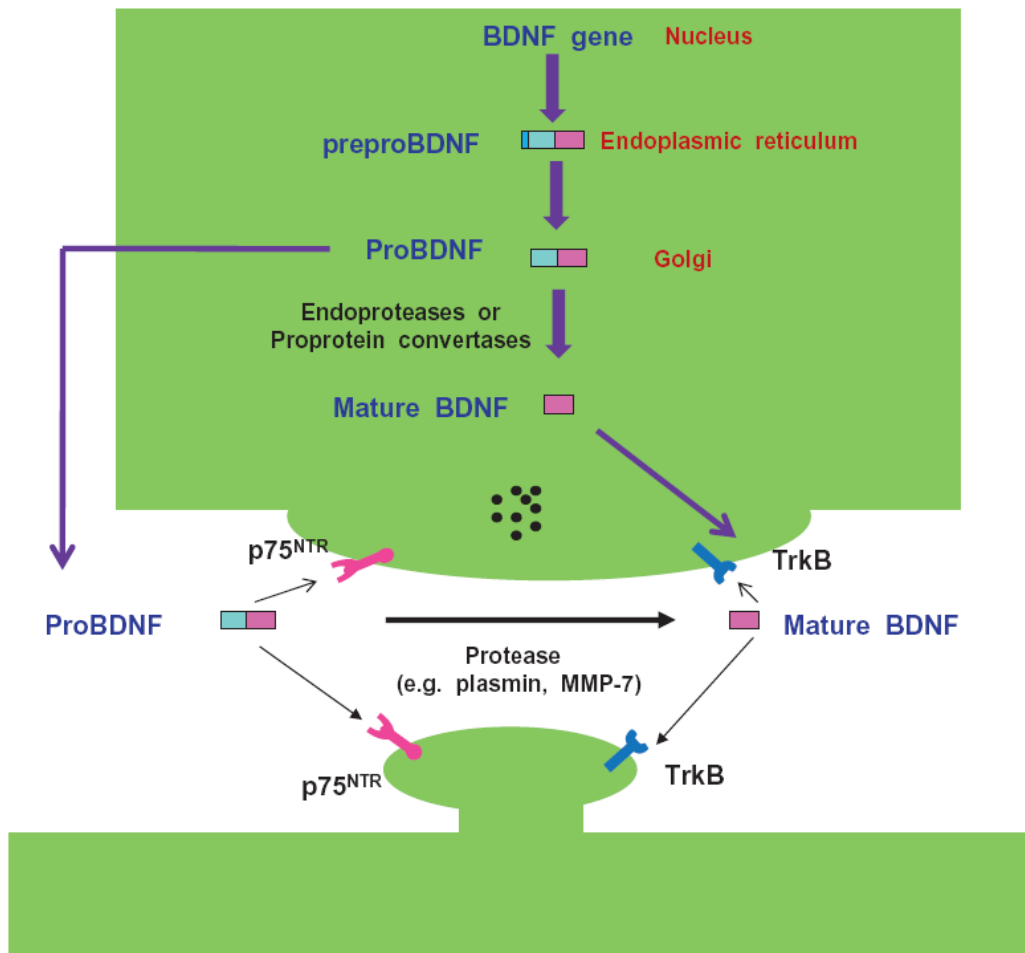


Figure 4. The synthesis of brain-derived neurotrophic factor (BDNF) from proBDNF. The BDNF gene produced precursor protein BDNF (preproBDNF) in the endoplasmic reticulum. ProBDNF binds to intracellular sortilin in the Golgi to facilitate proper folding of the mature domain. ProBDNF preferentially binds p75^{NTR} receptors. ProBDNF is cleaved by proteases (e.g. plasmin, matrix metalloproteinase [MMP]-7) at the synapses and converted to mature BDNF. Mature BDNF preferentially binds tropomyosin receptor kinase (Trk) B receptor.

In this regard, we decided initially to perform a systematic review and a meta-analysis of studies measuring serum concentrations of BDNF to elucidate whether or not this neurotrophin is abnormally produced in patients with schizophrenia. Additionally, we were interested in identifying factors that might contribute to the

different findings in literature, as to improve the design of future investigations in this field.

In fact, schizophrenia is a common severe psychiatric disorder which affects approximately 1% of the world population. Imaging studies and postmortem analysis have clearly shown that schizophrenia is not a mere functional disorder, but rather includes several histological abnormalities in various areas of the brain. Today, deficits in brain development or a malfunction in the dopaminergic system are considered the leading hallmarks of schizophrenia (Fatemi & Folsom, 2009; Howes & Kapur, 2009; Iritani, 2007). Although the pathogenesis of schizophrenia remains still unresolved, it is now clear that this disorder is the result of a complex interplay between inheritable genetic mutations in a large number of genes (a few common mutations with a small effect combined with many rare ones with a stronger effect), various environmental influences and epigenetic effects (van OS & Kapur, 2009; Owen et al., 2009; O'Donnell et al., 2009; Psychiatric GWAS Consortium [PGC], 2009; Roth et al., 2009). Over the years, multiple theories have been proposed to explain how these factors may generate schizophrenia. The different models proposed include principally the neurodevelopmental and the dopaminergic hypotheses which have been reviewed elsewhere (Fatemi & Folsom, 2009; Howes & Kapur, 2009). These two hypotheses may not necessarily be mutually exclusive as, for instance, a local dysfunction in dopaminergic neurotransmission may be the result of a failed development. Moreover, a number of studies pointed to the role of neurotrophins in the pathogenesis of schizophrenia. Neurotrophins are a small group of secreted dimeric proteins that affect the development of the nervous system in all vertebrates' species and are involved in the development and maturation of several brain networks including the dopaminergic system (Buckley et al. 2007; Shoval &

Weizman, 2005; Thome et al. 1998). Brain-Derived Neurotrophic Factor (BDNF) is the most widely distributed neurotrophin in the central nervous system (CNS) and is known to exert growth and trophic effects able to support many aspects of neuronal development including axonal growth and connectivity (Segal et al., 1995), neuronal survival and apoptosis (Segal et al., 1997), and formation of dopaminergic-related systems. Furthermore, BDNF has a dynamic effect on synaptic organization, promoting long-term changes of synaptic transmission (Shen et al., 1997), as well as learning and memory processes (Yamada et al., 2002). For these reasons many studies investigated the role of BDNF in the pathophysiology of schizophrenia but their findings resulted contradictory. For example, some postmortem studies conducted on schizophrenia brains showed elevated BDNF levels in the anterior cingulate, hippocampus (Takahashi et al., 2000) and cerebral cortex (Durany et al., 2001), whereas others found decreased BDNF levels in the hippocampus (Durany et al., 2001) and prefrontal cortex (Weickert et al., 2003, 2005). Interestingly, in both humans and rodents, BDNF is present not only in the brain but also in peripheral tissues and especially, in the blood (Pruunsild et al., 2007; Aid et al., 2007). The origin of circulating BDNF has been debated as this neurotrophin is produced by many different body tissues and epithelia, including smooth muscle cells of blood vessels (Donovan et al., 1995). However, it has been demonstrated that radiolabeled BDNF injected in the jugular vein or in the brain ventricle readily crosses the blood-brain barrier in both directions (Pan et al., 1998) and can be taken up by platelets that function as storage and release system (Karege et al., 2005). In addition, it has been shown that physical exercise induces an increase of serum BDNF levels which is contributed by 70% from the brain (Rasmussen et al., 2009). Thus, measurement of circulating BDNF is very attractive, because it may provide information on brain

functioning and blood samples are largely available and may be drawn non-invasively from living subjects as frequently as necessary. BDNF can be measured using simple enzyme linked immunoadsorbent assays (ELISA) that are commercially available and recent methodological studies have pointed out the possibility to obtain reliable measures of BDNF in serum preparations with stable values over several months of serum storage at 20°C, while in contrast, there is high variability in the measures of BDNF in whole blood or plasma because of the presence of release from platelets and degradation processes that are active even during storage (Elfving et al, 2009; Trajkovska et al., 2007). For these reasons, there is currently a great interest to validate the use of serum BDNF as possible biomarker in brain diseases, including psychiatric illnesses (for a recent meta-analysis of serum BDNF in depression see: Sen et al., 2008). To assess if BDNF can represent a good biomarker in schizophrenia, a growing number of studies compared BDNF serum levels between patients with schizophrenia and healthy control subjects but unfortunately, with controversial results. Indeed, several investigators found a significant decrease in serum BDNF concentrations (Carlino et al., 2011; Chen et al., 2009; Grillo et al., 2007; Ikeda et al., 2008, Jindal et al., 2010; Pirildar et al., 2004; Rizos et al., 2008; Shimizu et al., 2002; Tan et al., 2005a, 2005b; Toyooka et al., 2002; Xiu et al., 2009; Zhang et al., 2007, 2008); while other studies documented normal (Shimizu et al., 2003; Jockers-Schrubl et al. 2004; Huang et al. 2006) or even increased circulating BDNF (Gama et al., 2007; Reis et al., 2008).

3. Methods

3.1 Search strategy

The PUBMED, OVID MEDLINE, PSYCHINFO and EMBASE databases were searched using the following medical subject headings (MeSH): “Brain-Derived Neurotrophic Factor” OR State of Art of Serum Brain-Derived Neurotrophic Factor in Schizophrenia “BDNF” AND “schizophrenia”. In addition, all reference lists of the selected papers were examined for studies not indexed electronically. The search aimed to find all papers published through January 2011. We used the PRISMA guidelines to carry out this review (Figure 5).

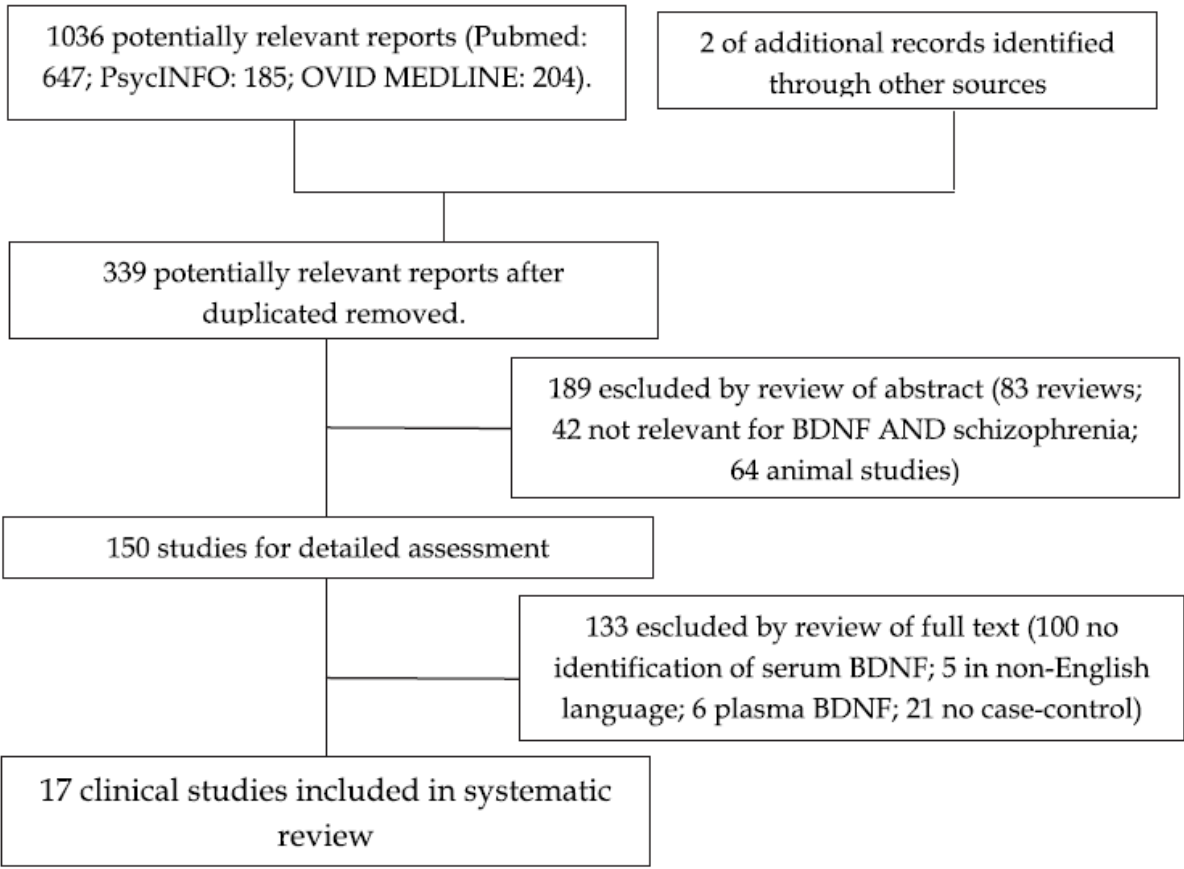


Figure 5. Flowchart of results of systematic review and meta-analysis search strategy.

3.2 Inclusion/exclusion criteria for both the systematic review and meta-analysis

Studies had to fulfill the following inclusion criteria :

1. Investigation of serum BDNF levels in patients with schizophrenia and healthy comparison subjects.
2. Mean serum BDNF reported (ng/ml or pg/ml).
3. Clinical characterization of patients with schizophrenia according to DSM-IV, ICD-10 or an equivalent system employed as a diagnostic tool. Study samples including some schizoaffective or schizophreniform subjects were also considered.
4. Published in English.

Exclusion criteria comprised:

1. Samples including non-schizophrenia psychosis or other schizophrenia spectrum disorders.
2. Plasma BDNF levels were measured.
3. Publications describing case reports or case series.
4. Patients or comparison subjects with neurological or medical disorders or substance or alcohol abuse.
5. Comparison subjects screened for psychiatric disorders.

In addition, in the studies exploring the same subject population or part of it, only the publication with the largest sample size was selected. When necessary, study Authors were contacted and asked to supply for missing or incomplete information.

3.3 Data abstraction and quality rating

Each paper was scrutinized by two independent reviewers (D.C. and M.B.) separately, and the following data from the article was obtained: age, gender, education, age at onset, duration of illness, number of hospitalizations, medications used (type, dosage and duration of treatment) and laboratory parameters. Mean serum BDNF levels (expressed in ng/ml) and the methods used for statistical analyses were also extracted from the article. Furthermore, the reviewers rated the quality of each study using a modified version of the quality rating check-list reported on Baiano et al. (2007).

Category 1: subjects

1. Prospective evaluation of patients, use of specific diagnostic criteria and description of demographic data;
2. Prospective evaluation of healthy control subjects, description of demographic data, exclusion of psychiatric and medical illnesses;
3. Presentation of significant variables (e.g. age, gender, age at onset, duration of illness, number of hospitalizations, medications used);

Category 2: methods for sampling and analysis

1. Clear description of laboratory technique and measurements, such in a way to be reproducible;
2. Blindness of investigators to experimental setup;
3. Report of intra and inter-assay reliability;

Category 3: results and conclusions

- Use of appropriate statistical tests;
- Presentation of main results and parameters for statistical significance;

- Consistence of conclusions with the results and discussion of study limits.

Each item was scored 1, 0.5 or 0 if criteria were completely met, partly met or unmet, respectively. This procedure was performed to evaluate the completeness of the available publications and not to criticize the investigations *per se*.

Authors	ELISA kit	Quality of rating (QR)	Control	Schizophrenic patients	
			Serum BDNF (mean ng/ml \pm SD)	Serum BDNF (mean ng/ml \pm SD)	Medication
Toyoka et al., 2002	Sigma Chemical	6.5	11.4 \pm 7.7	6.3 \pm 3.4	29=haloperidol, 5=chlorpromazine, 31=levomepromazine, 2=zotepin, 3=bromperidol, 1=risperidone, 9=other
Ikeda et al., 2003	Promega	9	52.2 \pm 25.3	37.1 \pm 20.4	Typical and atypical (dose not available)
Shimizu et al., 2003 (part I)	Promega	6.5	28.5 \pm 9.1	27.9 \pm 12.3	----
Shimizu et al., 2003 (part II)		6.5	28.5 \pm 9.1	23.8 \pm 8.1	
Pirildar et al., 2004 (part I)	Promega	8	26.8 \pm 9.3	14.4 \pm 2.8	17=risperidone, 2=clozapine, 3=olanzapine
Pirildar et al., 2004 (part II)		8	26.8 \pm 9.3	16.3 \pm 4.0	
Jockers-Schröbl et al., 2004	Promega	6.5	13.2 \pm 5.2	13.1 \pm 5.9	----
Tan et al., 2005	BanDing Biomedical	9	9.1 \pm 4.3	5.8 \pm 2.1	38=clozapine, 19=risperidone, 12=haloperidol, 5=chlorpromazine, 5=perphenazine, 2=others (<i>n</i> = 2)
Huang et al., 2006	Promega	5.5	14.17 \pm 6.9	14.2 \pm 6.9	----
Grillo et al., 2007	Chemicon	8	0.17 \pm 0.0	0.11 \pm 0.1	20=Clozapine; 24=typical antipsychotics; 6=chlorpromazine; 15=levomepromazine; 5=haloperidol

Authors	ELISA kit	Quality of rating (QR)	Control	Schizophrenic patients	
			Serum BDNF (mean ng/ml \pm SD)	Serum BDNF (mean ng/ml \pm SD)	Medication
Zhang et al., 2008	BanDing Biomedical	7.5	9.4 \pm 4.4	7.0 \pm 3.1	98=clozapine, 36=risperidone, 20=perphenazine, 19=haloperidol, 14=chlorpromazine, 9=fluphenazine, 9=trifluoperazine
Reis et al., 2008	R&D Systems	6	4.31 \pm 2.1	7.75 \pm 1.9	28=haloperidol, 3=chlorpromazine, 3=levoepromazine, 6=trifluoperazine
Rizos et al., 2008	R&D Systems	7	30.0 \pm 8.4	23.9 \pm 6.0	----
Xiu et al., 2009	BanDing Biomedical	9	11.9 \pm 2.3	9.9 \pm 2.0	157=Clozapine, 89=risperidone, 31=haloperidol, 21=chlorpromazine, 26=perphenazine, 27=sulpiride, 13=other
Chen et al., 2009	BanDing Biomedical	8.5	12.1 \pm 2.2	9.0 \pm 4.2	----
Jindal et al., 2010	Promega	9	116.78 \pm 38.42	97.58 \pm 31.41	----
Carlino et al., 2011	Promega	9	26.5 \pm 4.22	25.3 \pm 3.71	4=haloperidol, 2=zuclopenthixol, 2=haloperidol decanoate, 10= olanzapine, 6=risperidone, 8=quetiapine, 1=olanzapine +zuclopenthixol , 2=olanzapine +haloperidol, 2=quetiapine +haloperidol,2= quetiapine +zuclopenthixol, 3=risperidone+haloperidol

Table 1. Methodological aspects of the studies measuring serum BDNF in schizophrenia.

3.4 Statistical analysis

The calculations were performed by means of the statistical software package STATA 8.0 (StataCorp LP, Texas). Data were analyzed by using a random effects model (*Metan command*), which typically takes into account the between study variability, leading to wider confidence intervals than those obtained by a fixed effects model. Thus, studies were weighted for the inverse variance, obtaining the DerSimonian-Laird's effect size (Deeks et al., 2001). Heterogeneity between studies was explored using the Q-test. Since we hypothesized a statistically significant heterogeneity, a meta-regression analysis was planned to assess the effects of selected factors (i.e.: gender distribution, ethnicity, ELISA kit used and average age) on results between studies (*Metareg command*). Publication bias was assessed by Egger's tests (Egger et al., 1997) (*Metabias command*). All p values were two sided and the cutoff for statistical significance was 0.05.

4. Results

4.1 Systematic review

A total of 334 references were obtained. All the studies found in PUBMED database overlapped with those retrieved using OVID MEDLINE and PSYCHINFO databases. A total of 1036 references were identified. All the studies found in PUBMED database overlapped with those retrieved using OVID MEDLINE, EMBASE, PSYCHINFO lists. Most of them (322) did not meet the inclusion criteria, most analyzing *val66met*

BDNF polymorphism, mRNA expression or post-mortem studies. Thus, 17 were finally considered but 16 were actually included in the systematic review (Carlino et al., 2011; Chen et al., 2009; Gama et al., 2007; Grillo et al., 2007; Huang et al., 2006; Ikeda et al., 2008; Jindal et al., 2010; Jockers-Schrübl et al., 2004; Pirildar et al., 2004; Reis et al., 2008; Rizos et al., 2008; Shimizu et al., 2003; Tan et al., 2005a; Toyooka et al., 2002; Xiu et al., 2009; Zhang et al., 2008). Indeed, as per Authors suggestion, we excluded the study by Zhang et al. (2007) (124 patients and 50 controls) since the patients' sample consistently overlapped with that of the study published by Zhang and co-workers in the 2008 (196 patients and 50 controls).

Authors	Stages of disease	Ethnicity *	Control			Schizophrenic patients			Illness duration (mean-months \pm SD)
			N	Age (mean \pm SD)	M/F	N	Age (mean \pm SD)	M/F	
Toyoka et al., 2002	Chronic	JPT	35	45.6 \pm 11.3	14/21	34	48.6 \pm 14.0	17/17	25 \pm 12.3
Ikeda et al., 2003	Chronic	JPT	87	39.8 \pm 10.7	47/40	74	41.9 \pm 11.1	39/35	19.6 \pm 11.2
Shimizu et al., 2003 (part I)	Chronic	JPT	40	36.5 \pm 11.3	20/20	25	36.0 \pm 13.2	13/12	Med scz: 14.1 \pm 9.87; FEP: 1.09 \pm 1.36
Shimizu et al., 2003 (part II)	First episode/ drug naïve	JPT	40	36.5 \pm 10.7	20/20	15	34.7 \pm 16.0	7/8	
Pirildar et al., 2004 (part I)	Chronic	CEU	22	25.7 \pm 5.8	7/15	12	29.8 \pm 9.3	5/7	15.2 \pm 13.04
Pirildar et al., 2004 (part II)	First episode/ drug naïve	CEU	22	25.7 \pm 5.8	7/15	10	25.1 \pm 9.1	2/8	
Jockers-Schröbl et al., 2004	First episode/ drug naïve	CEU	61	32.3	28/33	102	33.3	50/52	----
Tan et al., 2005	Chronic	CHB	45	45.6 \pm 6.3	34/11	125	18.3 \pm 6.3	93/32	22.6 \pm 7.7
Huang et al., 2006	Chronic	CHB	96	29.1 \pm 10.0	36/60	126	34.0 \pm 10.0	72/54	6.0 \pm 5.0
Grillo et al., 2007	Chronic	YRI/CEU (ratio not specified)	25	34.1 \pm 7.2	12/13	44	35.5 \pm 7.2	19/25	

Authors	Stages of disease	Ethnicity *	Control			Schizophrenic patients			
			N	Age (mean \pm SD)	M/F	N	Age (mean \pm SD)	M/F	Illness duration (mean-months \pm SD)
Zhang et al., 2008	Chronic	CHB	50	----	34/16	196	----	130/66	22 \pm 7
Reis et al., 2008	Chronic	YRI/CEU (ratio not specified)	20	----	20/0	40	52.3 \pm 9.8	40/0	32.4 \pm 9.2
Rizos et al., 2008	First episode/ drug naïve	CEU	15	26.6 \pm 5.8	6/9	14	25.4 \pm 5.8	10/4	----
Xiu et al., 2009	Chronic	CHB	323	50.9 \pm 9.2	228/95	364	51.3 \pm 9.2	281/83	27.0 \pm 10.1
Chen et al., 2009	First episode/ drug naïve	CHB	90	29.9 \pm 9.8	49/41	88	29.2 \pm 9.6	47/41	23.4 \pm 19.1
Jindal et al., 2010	First episode/ drug naïve	CEU	41	22.31 \pm 5.67	25/16	24	22.4 \pm 5.47	17/10	----
Carlino et al., 2011	Chronic	CEU	40	46.78 \pm 10.79	20/20	40	49.23 \pm 9.03	20/20	23.05 \pm 10.99

Table 2. Clinical characteristics of studies included in the meta-analysis. International HapMap Project: YRI: Yoruba in Ibadan, Nigeria; JPT: Japanese in Tokyo; CHB: Han Chinese in Beijing, China; CEU: CEPH (Utah residents with ancestry from Northern and Western Europe).

4.1.1 Findings

Most of the studies (12/16) measuring serum BDNF documented lower concentrations of this neurotrophin in patients with schizophrenia (Carlino et al., 2011; Chen et al., 2009; Grillo et al., 2007; Ikeda et al., 2008; Jindal et al., 2010; Jockers-Schröbl et al., 2004; Pirildar et al., 2004; Rizos et al., 2008; Tan et al., 2005a; Toyooka et al., 2002; Xiu et al., 2009; Zhang et al., 2008); however, in other studies, BDNF concentrations were either increased (Gama et al., 2007; Reis et al.,

2008); or normal (Huang et al., 2006; Shimizu et al., 2003) (Table 1). Five out of the 16 researches investigated gender effects, demonstrating either significantly lower (Xiu et al., 2009) or higher serum BDNF levels in males suffering from schizophrenia (Gama et al., 2007). Conversely, no gender effect emerged in Carlino et al., (2011), Huang et al. (2006) and Rizos et al. (2008) and in all healthy control subjects.

4.1.2 Clinical features of patients

15/16 studies reported on the mean age of the patients with schizophrenia (mean: 37.22±9.48 SD years; range: 22.4-52.3). Nine studies provided data for age of onset of schizophrenia (mean: 25.70±4.86 SD years; range: 19.93-33.8) and 13 for length of illness (mean: 180.13±127.65 SD months; range 8.8-388.8). Six out of 16 papers reported on the mean dosage of antipsychotic medications, expressed as chlorpromazine equivalents (mean: 581.12±219.90 SD; range:330.4-936.6). In one paper (Zhang et al., 2008), data for other psychopharmacological treatment (lithium, valproic acid) were included, but there were no details about the role of these drugs on serum BDNF levels. Only Jockers- Schröbl et al. (2004) evaluated the role of substance abuse (cannabis) in serum BDNF levels: the Authors found significantly elevated BDNF serum concentrations (by up to 34%) in patients with chronic cannabis abuse or multiple substance abuse prior to disease onset. Drug-naïve schizophrenic patients without cannabis consumption showed similar results to normal controls and cannabis controls without schizophrenia. In relation to the source of recruitment, 6/16 studies included only inpatients (Chen et al., 2009; Reis et al., 2008; Rizos et al., 2008; Tan et al., 2005a; Xiu et al., 2009; Zhang et al., 2008), 1/16 included only outpatients (Gama et al., 2007), 3/16 considered both in-patients and

out-patients (Carlino et al., 2011; Huang et al., 2006; Ikeda et al., 2008) and 6/16 publications did not provide data (Grillo et al., 2007; Jindal et al., 2010; Jockers-Schrübl et al., 2004; Pirildar et al., 2004; Shimizu et al., 2003; Toyooka et al., 2002). In our research, we found that Japanese subjects were investigated in three studies (Ikeda et al., 2008; Shimizu et al., 2003; Toyooka et al., 2002), Caucasians in another four studies (Carlino et al., 2011; Jockers-Schrübl et al., 2004; Pirildar et al., 2004, Rizos et al., 2008) and Asians in five studies (Chen et al., 2009; Huang et al., 2006; Tan et al., 2005a, Xiu et al., 2009, Zhang et al., 2008). In four studies, ethnicity was unspecified (Gama et al., 2007; Grillo et al., 2007, Reis et al., 2008; Jindal et al., 2010). Six studies reported no diagnostic subtypes of schizophrenia (Ikeda et al., 2008; Gama et al., 2007; Jockers-Schrübl et al., 2004; Pirildar et al., 2004; Reis et al., 2008; Rizos et al., 2008; Toyooka et al., 2002) while in seven studies it was assessed the differences in serum BDNF levels among diagnostic subtypes (Chen et al., 2009; Grillo et al., 2007; Huang et al., 2006; Shimizu et al., 2003; Tan et al., 2005a; Xiu et al., 2009; Zhang et al., 2008). Different forms of schizophrenia had no association with BDNF serum levels in 5 papers (Grillo et al., 2007, Shimizu et al., 2003; Tan et al., 2005a; Xiu et al., 2009; Zhang et al., 2008), while Chen et al. (2009) showed significantly higher BDNF levels in paranoid (10.4 ± 4.3 ng/ml) compared to undifferentiated (8.0 ± 3.9 ng/ml) and other combined subtypes (7.5 ± 4.1 ng/ml). Huang et al. (2006) showed that patients with catatonic schizophrenia had lower serum BDNF protein levels than patients with paranoid schizophrenia and residual schizophrenia. In a second step, phase of illness and use of antipsychotic drugs were considered. We found that four studies enrolled only drug-naïve first-episode patients (Chen et al., 2009; Jindal et al., 2010; Jockers-Schrübl et al., 2004; Rizos et al., 2008), eight studies recruited only chronic, medicated patients (Carlino et al., 2011;

Gama et al., 2007; Ikeda et al., 2008; Reis et al., 2008; Tan et al., 2005a; Toyooka et al., 2002; Xiu et al., 2009; Zhang et al., 2008) and two studies included medicated patients with unclear phase of illness (Grillo et al., 2007; Huang et al., 2006). Notably, the study by Pirildar et al. (2004) and Shimizu et al. (2003) investigated both chronically antipsychotic-treated and drug-naïve patients; in particular, in Pirildar et al. (2004) some first episode medicated subjects were included. All the 16 studies excluded patients with a history of neurological disease, physical illness, and alcohol or substance abuse. Clinical data are summarized in Table 2.

4.1.3 Serum BDNF concentrations and laboratory procedures

Among studies, laboratory procedures were comparable with some minor variations. Sera were centrifuged to eliminate the blood clot with a mild centrifugation at 2000, 3000 or 3500 rpm for 5-15min at room temperature or 15°C); then, they were stored frozen at -70/ 80°C until used. Serum BDNF concentrations were measured using sandwich ELISA assays (see Table 1 for full detail). Finally, all the studies except one (Gama et al., 2007) reported clearly on mean BDNF concentrations. Specifically, mean serum BDNF values were 14.43 ± 10.24 SD ng/ml (range: 0.098-37.1) for patients and 17.99 ± 13.41 SD ng/ml, (range: 0.12-52.2) for healthy blood donors.

4.1.4 Study quality

The mean total quality scores for the reports were 7.63 ± 1.17 SD (min.:5.5; max.: 9). We correlated total and partial quality scores of studies on serum BDNF with the year of publication, which was significantly positively correlated to total quality score

($r=0.53$) and State of Art of Serum Brain-Derived Neurotrophic Factor in Schizophrenia study methodology score (Category 2) ($r=0.59$) but not to study design score (Category 1) ($r= 0.20$) or study consistency score (Category 3) ($r=0.27$).

4.1.5 Meta-analysis

Fifteen out of the 16 publications considered for the systematic review were used for the meta-analysis (Carlino et al., 2011; Chen et al., 2009; Grillo et al., 2007; Huang et al., 2006; Ikeda et al., 2008; Jindal et al., 2010; Jockers-Schrübl et al., 2004; Pirildar et al., 2004; Reis et al., 2008; Rizos et al., 2008; Shimizu et al., 2003; Tan et al., 2005a; Toyooka et al., 2002; Xiu et al., 2009; Zhang et al., 2008). The study by Gama et al., (2007) was eliminated, as it was the only one to report serum BDNF in $\text{pg}/\mu\text{g}$ of total protein while in all other studies serum BDNF concentration was given in ng or pg/ml serum and therefore, no comparison between the Gama's and the other studies was possible. Since the studies by Shimizu et al., (2003) and Pirildar et al., (2004) reported on separate data for both medicated and unmedicated patients, we performed calculations considering patients' subgroups as follows: Shimizu et al., 2003 part I and Pirildar et al., 2004 part I = medicated patients; Shimizu et al., 2003 part II and Pirildar et al., 2004 part II = unmedicated patients. Therefore, we carried out calculations on 17 samples of patients. Raw total serum BDNF levels (ng/ml) were used to calculate the related effect sizes. The overall estimate of SMD (*standardized mean differences*) in serum BDNF levels between patients with schizophrenia and healthy controls was significant ($z=4.14$; $p<0.001$) but considerable heterogeneity emerged from publications ($Q=139.15$; $d.f.=16$; $p<0.001$; $\tau^2= 0.2826$) (Figure 2). Therefore, we regressed the SMD against potential sources of

heterogeneity (i.e.: gender, age, ethnicity and ELISA kit used). This analysis demonstrated a significant association of BDNF levels with all these variables (age: $z=15.28$; gender: $z=10.92$ for males; $z=4.60$ for females; ethnicity: $Z=9.37$ and ELISA kit: $z=8.55$; $p<0.001$). Moreover, to determine if this systematic review and meta-analyses was subjected to publication bias (i.e. the presence of asymmetrical collection of data due to the missing of studies reporting negative results, or to the tendency of small studies to show greater effects than larger studies), we carried out the Egger's weighted regression and evidence of significant publication bias was found ($p<0.001$). Subsequently, the eleven studies investigating chronic, medicated patients with schizophrenia were considered (Carlino et al., 2011; Grillo et al., 2007; Huang et al., 2006; Ikeda et al., 2008; Pirildar et al., 2004 part I; Reis et al, 2008; Shimizu et al., 2003 part I; Tan et al., 2005a; Toyooka et al., 2002; Xiu et al., 2009; Zhang et al., 2008). Significant heterogeneity was found ($Q=120.85$, $d.f=10$, $p<0.001$, $\tau^2=0.3545$) and patients and healthy control subjects differed for serum BDNF levels, as demonstrated by the SMD test ($z=2.69$; $p=0.007$) (Figure 3). The meta regression analysis showed that age ($z=13.89$), gender ($z=11.09$ for males; $z=7.36$ for females), ethnicity ($z=6.76$), type of ELISA kit ($z=6.67$), chlorpromazine equivalents' medication dosage ($z=5.97$) and duration of illness ($z=7.03$) may all explain heterogeneity ($p<0.001$). Similar results were obtained by meta-analyzing the six studies including first episode unmedicated patients (Chen et al., 2009; Jindal et al., 2010; Pirildar et al., 2004; Rizos et al., 2008; Shimizu et al., 2003 part II) (Figure 4). Indeed, we found that both the Q test for heterogeneity ($Q=18.30$; $d.f =5$; $p=0.003$; $\tau^2=0.1595$) and Dersimonian and Laird pooled effect size ($z=3.06$, $p=0.002$) were significant. As previously demonstrated, heterogeneity may be due to age ($z=15.33$), gender ($z=4.55$ for males; $z=4.84$ for females), ethnicity ($z=5.47$), type of ELISA kit ($z=4.45$).

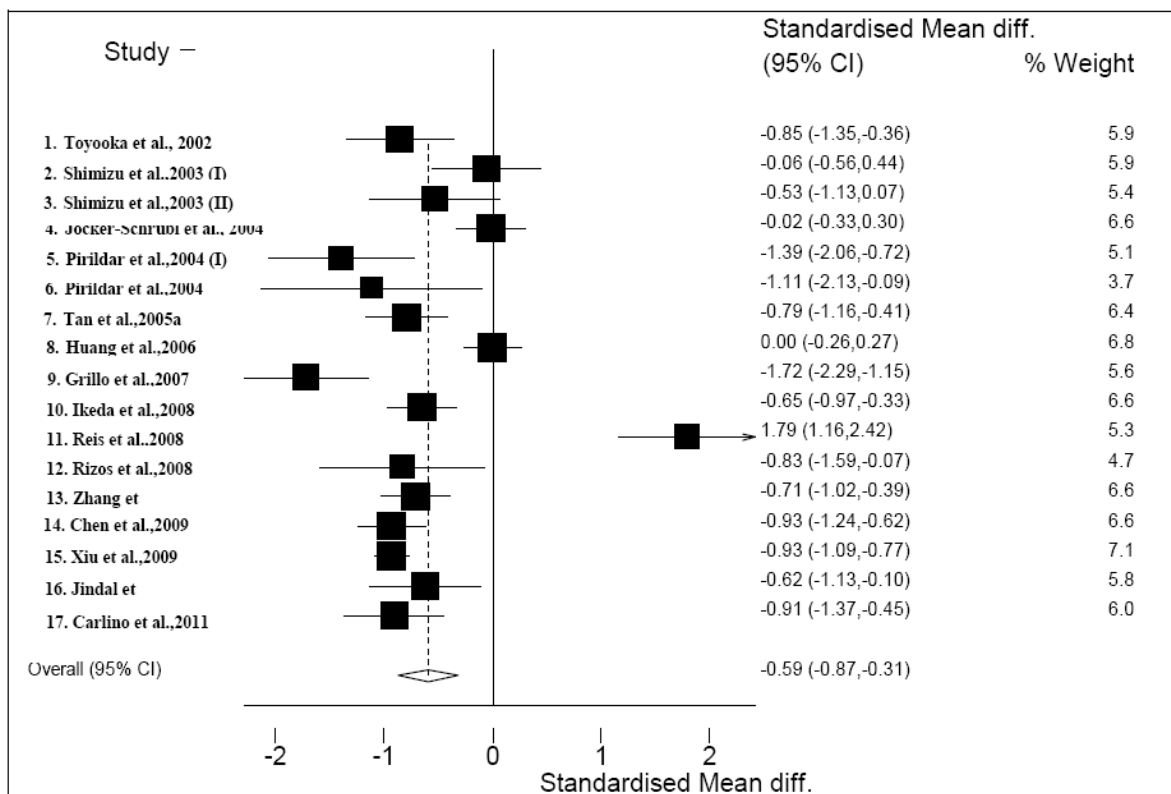


Figure 6. Forrest plot depicting the meta-analysis of serum BDNF levels in patients with schizophrenia.

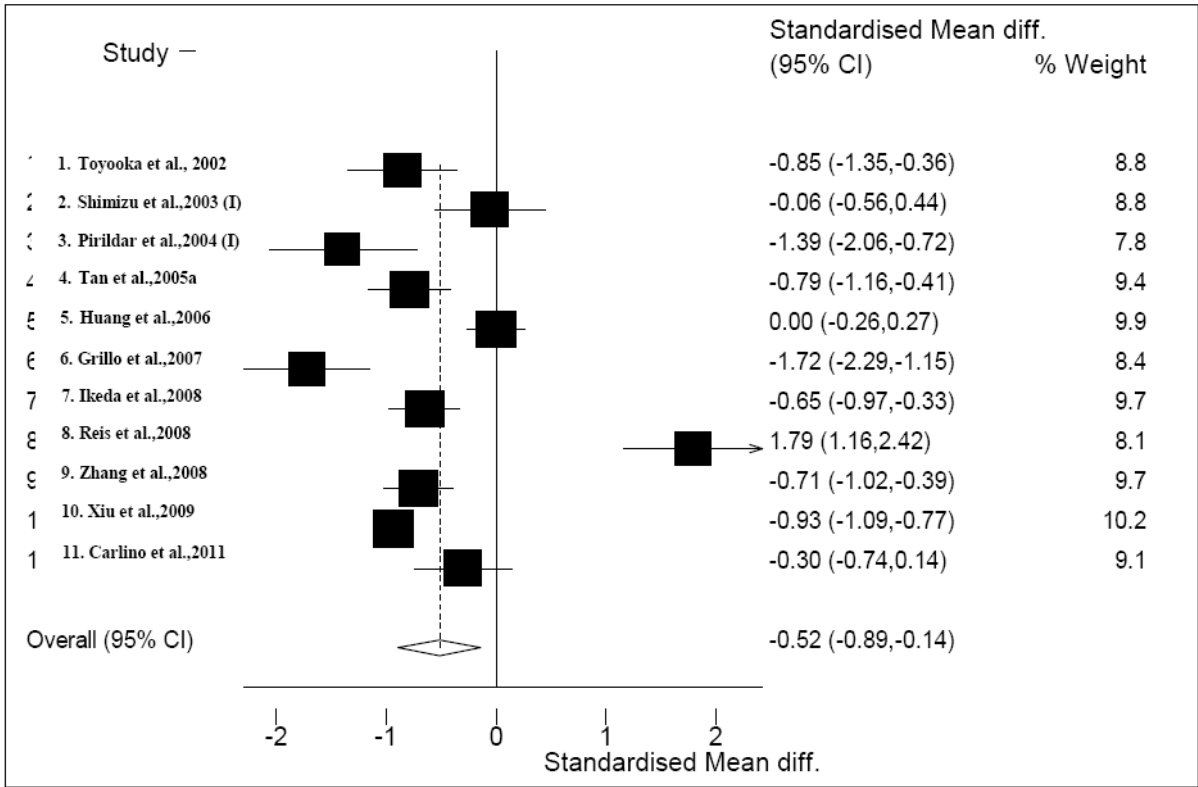


Figure 7. Forrest plot presenting the meta-analysis of serum BDNF in chronic medicated patients with schizophrenia.

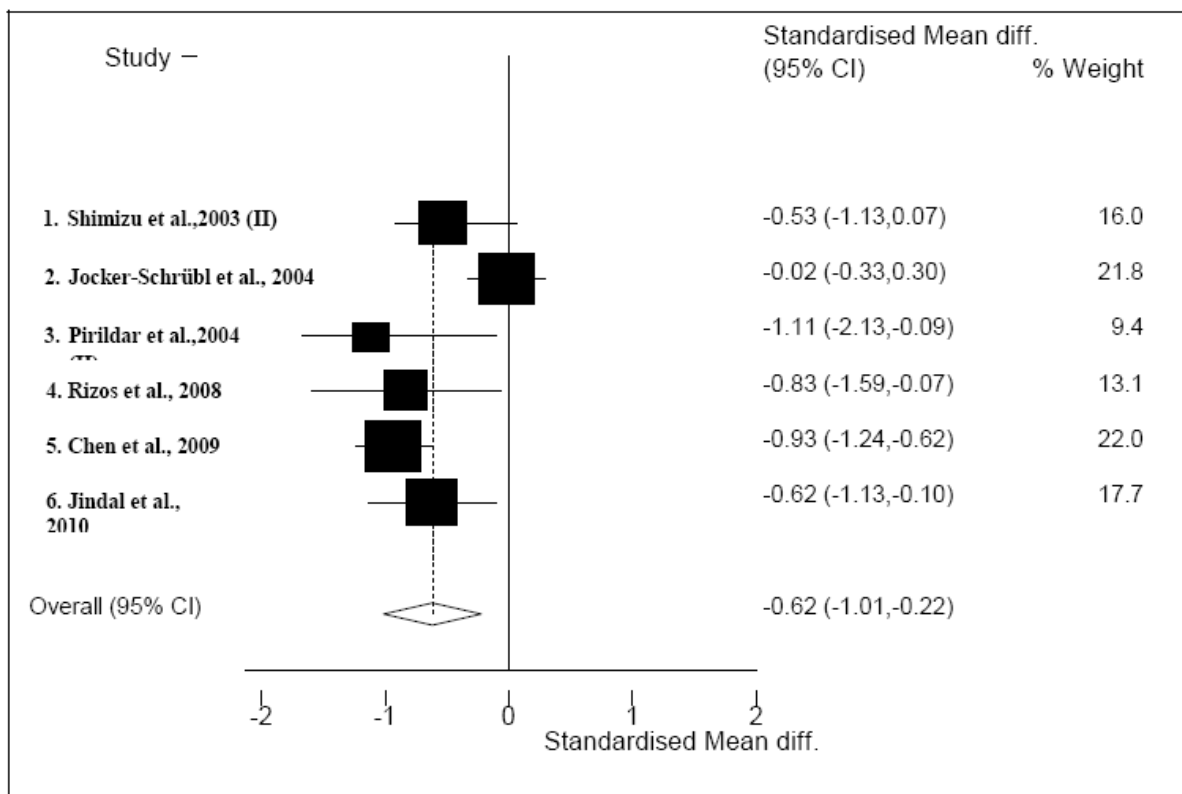


Figure 8. Forrest plot presenting the meta-analysis of serum BDNF in unmedicated patients with schizophrenia.

5. Conclusions of meta-analysis.

Our systematic review and meta-analysis showed that lower serum BDNF levels were detected in patients with schizophrenia in comparison to healthy controls in most even if not in all studies. Interestingly, reduced serum BDNF levels were found both in drug-naïve first episode and chronic medicated schizophrenia patients, as also found in studies investigating plasma BDNF concentrations (Buckely et al., 2007; Palomino et al., 2006; Tan et al., 2005b). These findings show that reduced serum BDNF levels are associated with schizophrenia but also suggest that serum BDNF is not a crucial biological marker of the clinical state in schizophrenia or a

marker of antipsychotic medication efficacy, in agreement with a recent meta-analysis (Green et al., 2010). The serum BDNF concentration in healthy populations, varied from a minimum of 0.17 ng/ml (Grillo et al., 2007) to a maximum of 52.2 ng/ml (Ikeda et al., 2003), the mean value among the thirteen paper analysed was 16.2 ng/ml (SD=14.59). Although we observed variability between studies using the same commercial ELISA, differences in serum BDNF concentration appeared mainly due to the different kit used. For example the three studies which used the kit purchased from BanDing Biomed reported values of serum BDNF in Chinese healthy donors of 11.9 + 2.3 ng/ml (Xiu et al., 2009); 9.4 + 4.4 ng/ml (Zhang et al., 2008); 9.1 + 4.3 ng/ml (Tan et al., 2005) while higher levels of serum BDNF were detected with the kit from Promega in healthy donors from Japan, 28.5 + 9.1 ng/ml (Shimizu et al., 2003); Turkey 26.8 + 9.3 ng/ml (Pirildar et al., 2004) and Taiwan 14.17 + 6.9 ng/ml (Huang et al., 2006). Finally, two studies using the R&D System ELISA assay showed very distant results because Rizos et al. (2008) detected 30.0 + 8.4 ng/ml of serum BDNF in healthy controls from Greece while Reis et al., (2008) in Brazil, found 4.31 + 2.1 ng/ml. Two other studies from Brazil also showed particularly low levels using the assay from another company (Chemicon), i.e. 0.19 + 0.1 ng/ml (Gama et al., 2007) and 0.17 + 0.0 ng/ml (Grillo et al., 2007). It is possible that this finding might reflect reduced amounts of serum BDNF in the Brazilian population. Another interpretation suggests a very low sensitivity of the ELISA kit from Chemicon. In conclusion, the most likely range of concentrations of serum BDNF in the World healthy population is 9-30 ng/ml with some possible specific regional variations. However, the great heterogeneity between studies and the presence of a publication bias may limit the interpretation of these results. Firstly, the phenotypic complexity, together with the multifarious nature of the so-called “schizophrenic psychoses”, limits our ability to

form a simple and logical, biologically-based hypothesis for the disease group. Secondly, all studies used ELISA assays that have different sensitivity and cannot distinguish between the three different protein forms of BDNF consisting in the precursor pro-BDNF (of 32 KDa) and its two proteolytic products mature BDNF (mBDNF of 14KDa) and truncated BDNF (truncBDNF of 28 KDa). Since pro-BDNF and mBDNF elicit opposing actions on synaptic plasticity and cell survival, their distinction could be essential to determine the role of BDNF in specific aspects of schizophrenia's neurobiology.

5.1 The role of proBDNF in patients with schizophrenia

BDNF is initially synthesized as a 32 KDa precursor protein (prepro-BDNF) in endoplasmic reticulum, and then processed into two isoforms (as the truncated-BDNF 28 KDa and mature 14 kDa BDNF) by two different proteolytic cleavages. Mature BDNF (mBDNF) is generated either intracellularly in the trans-Golgi by furin (Mowla et al., 2001, Matsumoto et al., 2008), or extracellularly by plasmin or matrixmetalloprotease-7 (Lee et al., 2001, Yang et al. 2009; Nagappan et al., 2009). Truncated-BDNF is cleaved by the Membrane Bound Transcription Factor Site-1 protease (MBTFS-1), also identified as Subtilisin/kexin isozyme 1 (Seidah et al., 1999). This isoform is not further processed into mBDNF and its function has not been elucidated yet. According to the "Ying and Yang" hypothesis (Lu et al., 2005), both mBDNF and proBDNF have particular neurobiological properties. In particular, proBDNF regulate neuronal survival (Teng et al., 2005; Koshimizu et al., 2009; Woo et al., 2005) and boosts synaptic pruning whereas mBDNF improves the differentiation of new neurons. Also, the conversion of proBDNF into mBDNF seems

to be decisive for signal transmission and synaptic plasticity. Indeed, mBDNF and the Tissue Plasminogen Activator (TPA) but not proBDNF are essential in late-phase long-term potentiation (L-LTP) and long-term memory (Pang et al., 2004).

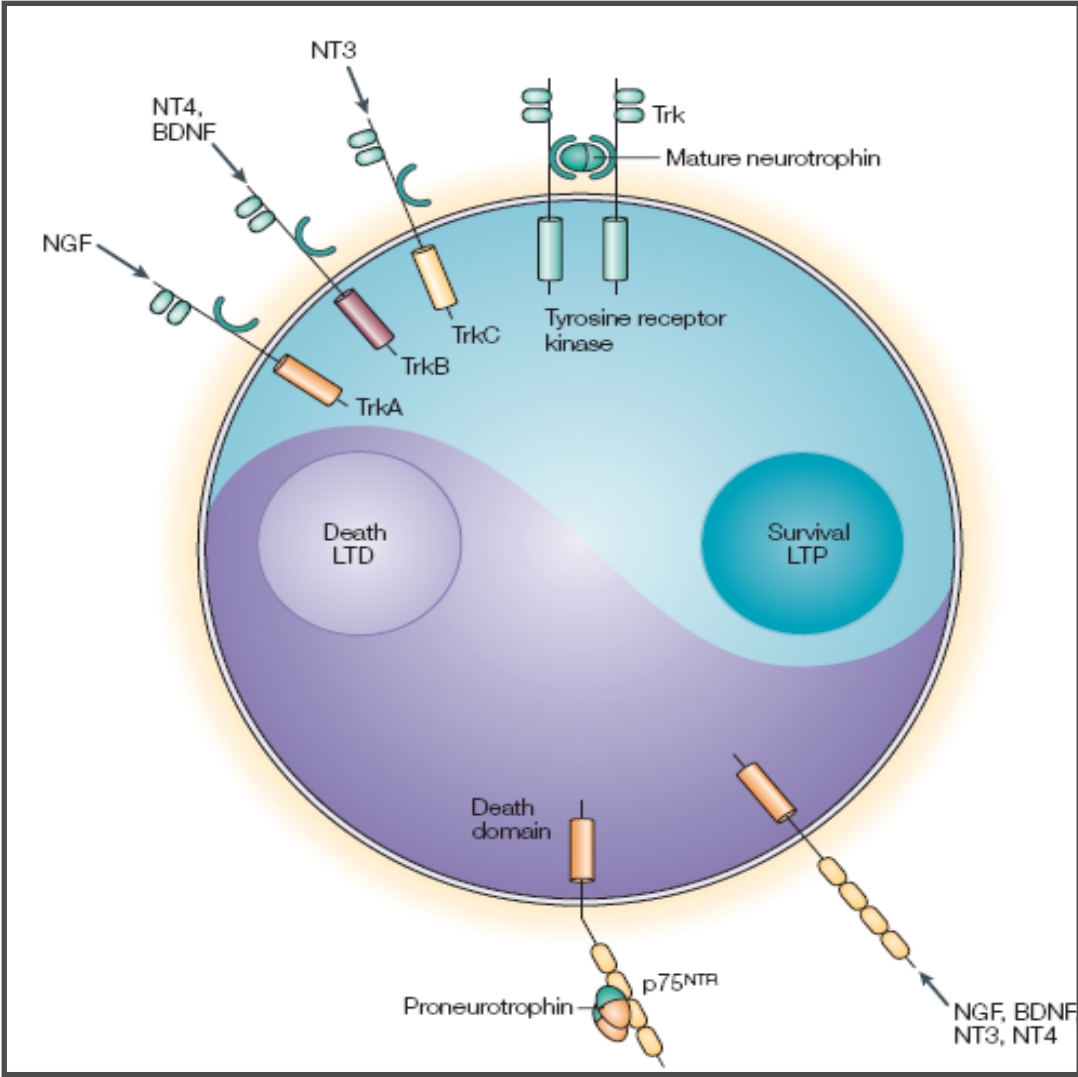


Figure 9. The ying-yang hypothesis

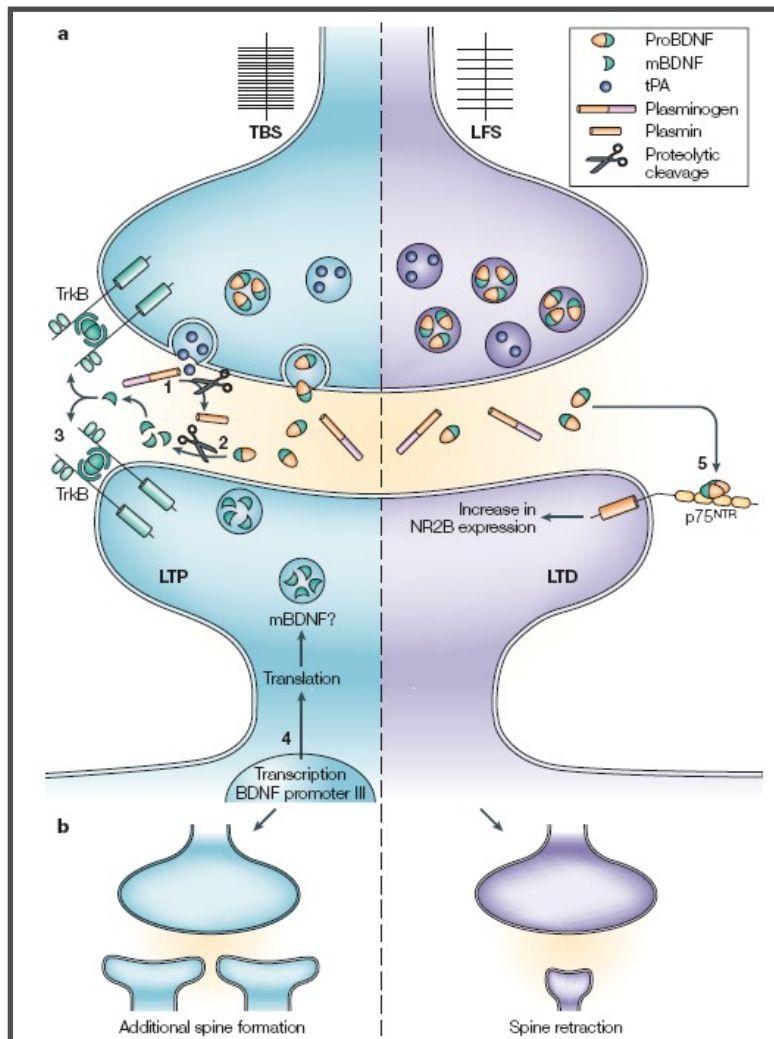


Figure 10. The ying-yang hypothesis and its relationship with long-term potentiation and long-term depression

These results underscore that a wrong matching of the proBDNF/mBDNF ratio may alter neuroplastic mechanisms, corresponding to the neurobiological substrate of impaired cognitive performance. An increasing number of postmortem researches have been carried out to measure the expression of proBDNF and mBDNF isoforms in animal models and in healthy human volunteers or subjects affected by neuropsychiatric disorders. Reduced mBDNF levels were found in three studies

(Karege et al., 2005; Weickert et al., 2003; Wong et al., 2010), particularly in the dorsolateral prefrontal cortex (DLPFC) of patients with schizophrenia compared to healthy controls. Weickert et al. (2003) found that mBDNF protein levels were not associated with post-mortem interval (PMI), tissue pH, age, or storage time of the serum. There were no significant main influences of gender or brain hemisphere, nor significant correlations between diagnosis and gender or diagnosis and brain hemisphere. On the other hand, Chen et al. (2001) showed that there was no significant variation for mBDNF among patients with schizophrenia and those with a diagnosis of affective disorders (unipolar and bipolar disorders) and Dunham et al., (2009) detected no difference for preproBDNF (35KDa) between patients with schizophrenia and those with unipolar depression and bipolar disorder. In contrast, Wong et al. (2010) found reduced truncated BDNF and preproBDNF proteins in the DLPFC of patients with schizophrenia, even if the reduction in preproBDNF protein did not achieve statistical significance. In a previous study (Carlino et al., 2011), we provided evidence of variation in serum levels of different BDNF isoforms in patients with chronic schizophrenia.

5.2 The role of ethnic differences in serum BDNF levels in patients with schizophrenia

An element of heterogeneity that emerges from this meta-analysis is represented by the ethnic differences amongst the samples. In this context it is of great interest to note that there are divergent findings of the positive or negative associations between BDNF *val66met* polymorphism and schizophrenia, especially in Caucasian and Asian participants. These differences may partially explain the differences in

serum BDNF levels among papers. Studies in *in vitro* and in animal models have shown that Met allele alters both sorting and secretion of proBDNF, such that less regulated (activity dependent) secretion is likely to occur in carriers of at least one Met allele. Several genetic associational studies have shown that SNPs in BDNF are associated with schizophrenia (Nanko et al., 2003; Szekeres et al., 2003), and a meta-analysis study also illustrated an association between C270T and schizophrenia (Zintzaras, 2007), but not between Val66Met and schizophrenia (Kanazawa et al., 2007; Naoe et al., 2007; Xu et al., 2007; Zintzaras, 2007). Great differences in the allelic frequencies for the BDNF Val66Met polymorphism between populations of different ethnic origins have been reported in public databases (<http://www.hapmap.org>) for the same populations (Tables 3 and 4). In Caucasian subjects, the frequency of the Met allele is 25–32%, whereas in Asian peoples the Met allele is more frequent, around 40–50% (Pivac et al., 2009; Verhagen et al., 2010). These variations among different ethnic groups in the allelic frequencies of the BDNF polymorphism may be caused by either the natural selection of an advantageous allele by unknown environmental issues or through a founder effect. However, we advise prudence in the analysis of these facts, also because despite this obvious difference in outcomes of schizophrenia across ethnicities, cross-cultural research in psychiatry focuses on similarities rather than differences. For example, subtypes of schizophrenia may have different prevalence across countries: in the International Pilot Study of Schizophrenia (1973) and the Determinants of Outcome of Severe Mental Disorders study (1992), catatonia was identified in 10% of cases in developing countries respect to less than 1% in developed countries. Hebephrenia was found in 13% of cases in developed countries and 4% in developing countries. Currently, we have not sufficient data about the role of diagnostic subtypes and

serum BDNF levels or BDNF polymorphisms. Another diagnostic caveat regards the Caucasian studies that often investigated not only patients with schizophrenia, but also subjects with schizophrenia spectrum disorders such as schizophreniform disorder or schizoaffective disorder, while Asian and other studies investigated only patients with schizophrenia. This difference in methodology might also have contributed to the inconsistent findings between the Caucasian and the Asian studies. The substantial variation in the Val66Met frequencies between Asian and Caucasian samples indicates that ethnicity may be of importance in the issue, because if the association among Caucasians reflects linkage disequilibrium with another gene variant, the extent of linkage may vary between populations. A recent study focused on the complex microsatellite polymorphism BDNF-LCPR located ~1.0 kbp upstream of the translation initiation site of BDNF (Okada et al., 2006); this polymorphism contained 23 novel allelic variants, including four major alleles (A1–A4). Kawashima et al. (2009) consider that if BDNF is indeed associated with schizophrenia, the A1 allele in BDNF LCPR would be a hopefully useful marker in the Japanese population. Also, we must not forget the interchange between genetic and environmental issues, that may essentially vary for men and women. In this regard, it would be interesting to evaluate if gender-related epistatic effects pertaining to the *Val66Met* polymorphism subsist. Literature data showed that gender differences in schizophrenia reproduce divergences in neurodevelopmental mechanisms and social influences on illness risk and course. Men have poorer premorbid functioning and have worse negative and less depressive manifestations than women. Substance abuse is more frequent in male. Results of gender variations in brain morphology (e.g. hippocampal volume) are conflicting but refer to matter of sexual dimorphism, meaning that the same elements are significant to explain sex disparities in both

normal neurodevelopment and those in relationship with schizophrenia. Another factor to consider is the epigenetic influence. “Epigenetic” refers to the covalent modifications of chromatin. Epigenetic machinery not only is responsible for lasting differences in gene activity in the CNS but also controls gene expression necessary for cognition. Thus, the likelihood of an epigenetic involvement in schizophrenia is an interesting hypothesis. In fact, epidemiological studies have identified several environmental risk factors for schizophrenia, counting marijuana consumption and obstetric complications. A recent study by Nicodemus and colleagues (2008) showed a significant association between four candidate genes for schizophrenia which are likely to have a role in hypoxic situations, including BDNF detecting significant evidence for gene x environment interaction in schizophrenic patients with or without obstetric complications. Recently, several studies underline that DNA methylation contributing to ongoing regulation of BDNF transcription in the CNS to control synaptic plasticity and memory mechanism (for e review, see Roth et al., 2009a). In addition, BDNF DNA methylation has also been found to play a part in altered gene expression in response to environmental pressure, such as social experiences (Roth et al., 2009). Indeed, stressful social experiences early in life have long-lasting consequences such as increased anxiety, drug seeking behavior, cognitive impairment, and altered affiliative behaviours (Branchi et al., 2004; Fumagalli et al., 2007; Lippmann et al., 2007). Finally, it was recently revealed that social experiences early during the first postnatal week generate lasting changes in DNA methylation in BDNF gene in relationship with reduction of BDNF gene expression in the adult prefrontal cortex (Roth et al., 2009b). Overall, the available data suggest that DNA methylation may indeed be an epigenetic mechanism that contributes to the aberrant regulation of genes associated with schizophrenia. The hard work to recognize

vulnerability genes for multifactorial disorders such as schizophrenia, has inspired the development of alternative methodologies. Since genetic heterogeneity has been a major dilemma in complex disorders, investigators have attempted to increase homogeneity in their samples. Recently, alternative phenotypic definitions have been defined that might be more closely linked to biological pathway (*endophenotypes*), for example sensory gating deficits or working memory dysfunction (Gottesman and Gould, 2003). Another advance to decrease basic genetic complexity is the utilization of genetic isolates. Isolated populations originated from a small number of founder couples. Throughout history, many populations, counting isolated as well as outbred populations, undergo alternating era of adversities (e.g. war, epidemics, or famine) with period characterized by rapid growth of the population. Due to increased inbreeding and genetic drift in isolates, certain alleles will be present more frequently in the population, while others are lost, increasing genetic homogeneity. Additionally, due to geographic, cultural, or religious barriers, isolated populations did not experience a large degree of admixture with adjacent peoples for many generations, ensuing in a relatively small gene pool (“founder effect”). So the recognition of a gene or allele that clinically and/or genetically is not as important in outbred populations as in isolated populations might untangle molecular pathways and find out new candidate genes, which might have a higher involvement on illness risk in general. Several studies highlight susceptibility loci for schizophrenia in isolated populations (Venken et al., 2007).

Genotype - Population descriptors	Genotype frequencies
G/G - YRI	0.683
G/G - JPT	0.190
G/G - CHB	0.488
G/G - CEU	1.000
A/G- YRI	0.283
A/G- JPT	0.357
A/G- CHB	0.349
A/G- CEU	n.a.
A/A- YRI	0.033
A/A- JPT	0.452
A/A- CHB	0.163
A/A- CEU	0

Figure 3. YRI: Yoruba in Ibadan, Nigeria; JPT: Japanese in Tokyo, Japan; CHB: Han Chinese in Beijing, China; CEU: CEPH (Utah residents with ancestry from northern and western Europe). G/G = Met/Met; A/G = Val/Met; A/A = Val/Val.

Allele - Population descriptors	Allele frequencies
G- YRI	0.825
G- JPT	0.369
G- CHB	0.663
G- CEU	1.000
A- YRI	0.175
A - JPT	0.631
A- CHB	0.337
A- CEU	0

Figure 4. YRI: Yoruba in Ibadan, Nigeria; JPT: Japanese in Tokyo, Japan; CHB: Han Chinese in Beijing, China; CEU: CEPH (Utah residents with ancestry from northern and western Europe).

6. Is altered BDNF proteolytic processing a general feature in patients with cognitive impairment?

6.1 Role of pro/matBDNF in animal models of learning and memory

The molecular mechanisms supporting the long-term fear memory (Rodrigues SM, et al., 2004; Costa-Mattioli M, et al., 2007; LaLumiere RT et al., 2005) are very similar to those involved in the memory formation in neuronal circuits, L-LTP (Raymond, 2007). Barnes et al. (2008) showed that reduced proteolysis of proBDNF in the hippocampus is fundamental to regulate protein synthesis-dependent extinction of contextual fear memory (CFM). Importantly, increasing endogenous proBDNF and reducing mBDNF levels in the CA1 supported extinction. On the contrary, the formation of CFM was associated with increased proteolytic processing of proBDNF. Therefore, the processing of BDNF was associated with the acquisition and updating of information related to salient stimuli, mediating changes in behaviour. Thus, BDNF regulates the acquisition, consolidation and extinction of fear memory, but not reconsolidation. Indeed, the tPA mediated proteolysis of proBDNF facilitates new learning but be in opposition to the extinction of strengthened memories. Several studies sustained that peripheral administration of lipopolysaccharide (LPS) injuring the LTP (Vereker et al., 2000; Hauss-Wegrzyniak et al., 2002; Kelly et al., 2003a,b; Bany et al., 2005) and other types of hippocampal-based learning (Shaw et al., 2001). Moreover, it was hypothesized that peripheral LPS somministration caused memory deficit in adult rats after perinatal programming (Gayle et al., 2004; Bilbo et al., 2005). The intraperitoneal administration of a single, high dose of endotoxin

decreased proBDNF- and BDNF protein levels at synaptic sites in adult mouse brains. These changes were associated with a reduction in BDNF-, CRH- as well as POMC (pro-opiomelanocortin) mRNA levels (Schnydrig et al., 2007). Since it was known that prenatal exposure to opiates, favored abnormal cognitive performance, Schrott et al. (2008) examined whether continuous exposure to the opiate *l*- α -acetylmethadol (LAAM) affected BDNF biosynthesis, as well as acquisition or retention of a complex learning task (i.e.the eight-arm radial maze) Rats prenatally exposed to LAAM had poorer cognitive performance than prenatal water-treated controls, although they were able to acquire the task by the end of training and there were no differences between the groups on retention 24 hr after testing. Also, proBDNF precursor protein was significantly decreased in the synaptic fraction of trained prenatal LAAM-treated rats compared to prenatal water-treated trained controls. In untrained rats, prenatal treatment did not affect any of the measures. Silhol et al. (2007) studied BDNF and proBDNF hippocampal levels in young (2-month-old) and aged (24-month-old) Wistar rats, before and after learning task training. Spatial memory was assessed by a water-maze procedure linking visible and invisible platform location learning. Under basal conditions, BDNF levels measured using ELISA assay was higher in the hippocampus of aged rats versus those of young rats. As expected, after the learning task the BDNF content was significantly increased in young rats but not in the same rats. Nevertheless, since it has been previously reported that the ELISA assay antibody recognizes other BDNF containing molecules (Fayard et al., 2005; Silhol et al., 2005), the BDNF content was analyzed more specifically by Western blot. Results substantially replicated those obtained using the ELISA assay; moreover, proBDNF levels were significantly increased after training both in young and aged rats. The same research group

(Silhol et al., 2008) determined the BDNF content by ELISA assay in Lou/C rats, an inbred strain of Wistar origin, which is a known animal model of successful aging with a longer lifespan and conserved memory abilities than other rat strains. The Authors showed that the hippocampus of young Lou/C rats had two-fold higher BDNF concentrations than that of young Wistar rats; additionally both young and aged Lou/C rats had higher amounts of BDNF and proBDNF contents. In aged Lou/C rats the decrease of hippocampal BDNF t detected by ELISA assay was associated with reduced proBDNF content, as BDNF levels remained unchanged in young and aged rats. In contrast, in aged Wistar rats the increase of BDNF content measured with ELISA assay was due to increased levels of the two peptides, i.e., BDNF (14-kDa band) and proBDNF (28-kDa band). Differences were noted in the dentate gyrus and CA1 layers, and aged Lou/C rats had less proBDNF-labeled cells in the superior blade of the dentate gyrus than young Lou/C rats. Conversely, aged Wistar rats had more labeled cells than young Wistar rats in this region. In the dentate gyrus inferior blade, no significant differences were observed between the two groups. A slight but significant difference was noted in the CA1 layer of aged Wistar rats that presented more labeled cells than young rats. It is known that the tolloid/bone morphogenetic protein-1 (BMP-1) family of metalloproteinases is involved in synaptic plasticity in human adults. To this date, Keifer et al. (2009) examined the role of a reptilian orthologue of the tolloid gene family (turtle tolloid-like gene or tTLL) in an *in vitro* model of eyeblink classical conditioning using an isolated brain stem preparation. Analysis by real-time RT-PCR showed that an extracellularly secreted form of tTLL(tTLLs), was momentarily expressed in the early phases of conditioning; furthermore, it was observed that tTLLs cleaved proBDNF into mBDNF in and expression of recombinant tTLLs protein induced mBDNF expression. mBDNF is

modestly expressed in cultures treated with anti-tTLL siRNA. Zheng et al., 2010 found that application of oligomeric Amyloid- β ($A\beta$) in another *in vitro* model of classical conditioning impaired acquisition of conditioning. This effect was associated with inhibition of mBDNF protein expression, suppressed phosphorylation of key signal transduction elements, and reduced AMPA receptor synaptic delivery. The oligomeric $A\beta$ appeared to affect the normal role of a learning-induced tTLLs, which cleaves proBDNF to mature BDNF. Therefore, the $A\beta$ -mediated BDNF reduction may be a secondary effect of its ability to move proteolytic processing away from its normal substrates during learning.

6.2 Altered processing of BDNF in patients with cognitive impairment

Peng et al. (2005) found that the amount of proBDNF (36 KDa) decreased 21 and 30% in mild cognitive impairment (MCI) and Alzheimer's Disease (AD) groups, respectively, as compared with healthy subjects, consistent with their previous results of a 40% decrease in end-stage AD (Michalski et al., 2003). mBDNF was reduced 34 and 62% in MCI and AD groups, respectively (Peng et al., 2005). Thus, the decrease of mature BDNF and proBDNF precedes the decline in choline acetyltransferase activity, which typically occurs later in AD. Both proBDNF and mBDNF levels were positively correlated with cognitive measures, as the Global Cognitive Score and Mini Mental State Examination score. Weickert et al. (2003) detected significantly reduced levels of mBDNF (by over 40%) in the dorsolateral prefrontal cortex (DLPFC) in patients with schizophrenia compared to normal controls. BDNF protein levels within the DLPFC did not correlate with the average daily dose of neuroleptics, the dose of neuroleptics most recently taken, the lifetime neuroleptic

exposure estimate, age of onset, and illness duration. These findings were replicated in the study carried out by Wong et al. (2010), with a 23% reduction in mBDNF immunoreactive levels in schizophrenic subjects. The expression of the ~32 kDa preproBDNF and ~28 kDa proBDNF protein was reduced by 14% and 10.4% in schizophrenics versus to controls respectively, although only the proBDNF protein reached the statistical significance. No significant correlation was found between mBDNF and age. Conversely, the Authors observed a significant positive correlation between proBDNF and age. Finally, BDNF protein expression in the DLPFC was not significantly correlated with duration of illness or any estimated measure of antipsychotic exposure. In support of these findings, Karege et al. (2005) reported that mBDNF was reduced in the DLPFC of a mixed group of patients including some patients with schizophrenia. In opposition while in the study of Duhnam et al. (2009) although in schizophrenia mean proBDNF (35 KDa) densities were lower than controls in most subregions, they did not reach significance.

Table 5*. Characteristics of each preclinical study who investigated precursor forms of BDNF.

Authors	Paradigm	Subregions dissected/neuronal culture	Animal model	Antibodies/chemicals	Principal findings
Schnydrig et al., 2007	Effects of a single i.p. injection of LPS on proBDNF-/BDNF protein expression	Crude synaptosomal preparations	C57BL/6J mice	Santa Cruz Biotechnology	Transient reductions in synaptosomal proBDNF- and BDNF protein expression. BDNF-, CRH- and POMC mRNA levels are also significantly reduced.
Silhol et al., 2007	Spatial learning in the water-maze	DG, CA1, CA2, CA3	Wistar young (2-month-old), aged (24-month-old) adult male rats	Santa Cruz Biotechnology	Training increased the proBDNF content in both young and aged rats which can probably be processed in BDNF.
Silhol et al., 2008		DG, CA1, CA2, CA3	Young (2 month-old) and aged Lou/C vs matched Wistar (23–25 months old) rats	Santa Cruz Biotechnology	Young and aged Lou/C rats had higher amounts of BDNF and proBDNF content than Wistar rats. proBDNF content was reduced in aged Lou/C rats and increased in aged Wistar rats.
Barnes et al., 2008	Fear conditioning	CA1	Adult male Lister hooded rats	Chemicon	Acquisition and extinction of CFM depend on the increased and decreased proteolysis of proBDNF in the hippocampus, respectively. Reconsolidation of CFM is not correlated with altered proBDNF cleavage.
Keifer et al., 2009	Eyeblink classical conditioning	The pons with the cerebellar circuitry removed	Pseudemys scripta elegans	Santa Cruz Biotechnology	Expression of an extracellularly secreted tolloid-like metalloproteinase is regulated in the early stages of classical conditioning and functions in the conversion of proBDNF to mBDNF.
Zheng et al., 2010	Classical conditioning	The pons with the cerebellar circuitry removed	Pseudemys scripta elegans	Santa Cruz Biotechnology	An A β -induced reduction in BDNF, due to interference in the proteolytic conversion of proBDNF to mBDNF.

*HFS=High Frequency Stimulation, LFS=Low Frequency Stimulation, A β =Amiloid-B, NPV=Negative Predictive Value; I-TBS: theta burst stimulation, IOP:intraocular pressure, HFS: high frequency stimulation, LES: low frequency stimulation, SSCs: spontaneous synaptic current, ESCs: evoked synaptic current, CR-proBDNF: cleavage resistant proBDNF, ko: knock out, ki: knock in, DCG: dendritic cell granule; MMPs= matrix metalloproteinases.

Table 6 *. Characteristics of each study who investigated precursor forms of BDNF in humans.

Authors	Sample Collection	Subjects	Neuropsychological assessment	Antibodies/ Chemicals	Principal Findings
Michalski et al., 2003	Postmortem parietal cortex samples	AD= 7; HC=8	MMSE	Santa Cruz Biotechnology	Reduction proBDNF 40% in parietal cortex
Weickert et al., 2003	Postmortem DLPFC	Schizophrenia	_____	Chemicon	A significant reduction in BDNF mRNA (mean = 23%) and protein (mean = 40%) in the DLPFC of patients with schizophrenia compared to normal individuals.
Peng et al., 2005	Postmortem parietal cortex samples	MCI=17; AD=17	MMSE, CGS	Santa Cruz Biotechnology	proBDNF decreased 21 and 30% in MCI and AD, respectively. mBDNF was reduced 34 and 62% in MCI and AD, respectively. proBDNF and mBDNF levels were positively correlated with cognitive measures such as the GCS and the MMSE score.
Dunham et al., 2009	Postmortem hippocampus samples	Schizophrenia, MDD, BPD	_____	Santa Cruz Biotechnology	In schizophrenia, although mean (pro)BDNF densities were lower than controls in most subregions, they did not reach significance.
Wong et al., 2010	Postmortem hippocampus, DLPFC, parietal cortex samples	Schizophrenic pts=71; hc=71	_____	Santa Cruz Biotechnology	BDNF protein expression is reduced in the DLPFC of patients with schizophrenia.
Carlino et al., 2010	Serum samples	Schizophrenic pts=40; hc=40	Trail Making Test A, B, Digit Span, Letter-Number Sequencing, Digit Symbol Coding subtests of WAIS. The IQ was determined using the short form of the WAIS. PANSS	Promega	A slight reduction in serum BDNF levels in SZ patients with respect to HC. Increased serum pro-BDNF and mat-BDNF and reduced truncated-BDNF in SZ with respect to HC. Patients with an increase in pro-BDNF or mat-BDNF higher than the HC mean + 2 SD also had >2SD reduction of truncated-BDNF. Reduced truncated-BDNF correlated significantly with higher positive and lower negative PANNS scores and a worst performance in all cognitive assays but not with antipsychotic type.

*PANSS=Positive And Negative Syndrome Scale, WAIS, NPV=Negative Predictive Value, PPV=Positive Predictive Value, MCI=Mild Cognitive Impairment, AD=Alzheimer Disease, NCI=No Cognitive Impairment, Pts=Patients, DLPFC= Dorsolateral Prefrontal Cortex, MMSE=Mini Mental State Examination, CGS=Global Cognitive Score; PSA-NCAM= polysialylated-neural cell adhesion molecule

7. Methods and materials

7.1. Subjects

The study was approved by the local Ethics Committee. Written informed consent was obtained from all participants or, their first degree relatives. Subjects were Caucasians chronic outpatients with a Diagnostic and Statistical Manual of mental disorders Version-IV (DSM-IV) diagnosis of schizophrenia and fulfilled the criteria for the Structured Clinical Interview for DSM disorders version-I (SCID-I). Patients with a history of substance or alcohol abuse, personality disorder, medical or neurological disease, dementia or mental retardation were excluded. Psychiatric symptoms were rated using the Positive and Negative Syndrome Subscale PANSS (Kay et al., 1989). Clinical subtypes of schizophrenia were as follows: residual, 9 (22.5%); paranoid, 10 (25.0%); catatonic, 2 (5.0%); hebephrenic, 1 (2.5%); disorganized, 6 (15.0%); undifferentiated, 12 (30.0%). The mean antipsychotic daily dose use was 424.6 – 270.4 (milligrams of chlorpromazine equivalents). The average duration of antipsychotic treatment was 4.47 ± 3.43 years at the time of investigation. Treatment consisted of monotherapy with atypical antipsychotic (n=24) or typical antipsychotics (n = 6), and 10 patients received multiple drugs simultaneously (typical ± atypical). Typical antipsychotics were: haloperidol (n=4), Zuclopenthixol (n=2), haloperidol decanoate (n=2). Atypical antipsychotics were: olanzapine (n=10), risperidone (n=6), quetiapine (n=8). The combined therapy consisted in: olanzapine ± Zuclopenthixol (n=1), olanzapine ± haloperidol (n=2), quetiapine ± haloperidol (n=2), quetiapine ± Zuclopenthixol (n=2), risperidone ± haloperidol (n=3). In addition 23 patients received one (n=15) or two (n=8) antiparkinsonian drugs. Other drugs taken by the patients

included antibiotics, antihypertensives and gastro-protectors, but not anticoagulants. Antidepressant and/or mood stabilizers were not used by this patient population. Forty healthy control Caucasians subjects matched to patients for age, gender and ethnicity were recruited. None presented a personal or family history of psychiatric disorder (SCID nonpatient edition).

7.2. Neurocognitive assessments

Processing speed, attention, executive function, and working memory were assessed by means of an abbreviated neuropsychological battery (Harvey et al., 2009) consisting in Trail Making Test Parts A and B, Digit Span, Letter-Number Sequencing, and Digit Symbol Coding subtests of the Wechsler Adult Intelligence Scale, 3rd edition (Wechsler, 1997). The intelligence quotient (IQ) was determined using the short form of the Wechsler Adult Intelligence Scale (WAIS), which includes vocabulary, math, picture arrangement, and block design.

7.3. Measurement of serum BDNF

Blood (5 ml) was collected between 8 and 9 h AM in anticoagulant-free tubes and maintained at RT for 1 h, followed by 1 h at 4 °C. After centrifugation at 2000 g for 10 min at 4 °C, serum samples were stored up to 2 months at -20 °C and then analysed contemporarily in triplicate. Sera were diluted 1:50 in sample buffer and BDNF was quantified using an ELISA kit (BDNF Emax immunoassay system, Promega) in a microplate reader (Anthos Labtec Instrument) set at 450 nm.

7.4. Western-blotting

Serum samples were depleted from albumin and IgGs using Qproteome Albumin/IgG Depletion Kit (Qiagen). Total proteins amount in purified serum samples was determined using Bradford assay (SigmaAldrich,). Proteins (30 mg) were separated on 12% SDS polyacrylamide gels and transferred onto nitrocellulose Protran membranes (Whatman). After blocking (4% non-fat milk powder, 0.05% tween-20 in phosphate-buffered saline) at RT, membranes were incubated overnight at 4 °C with one anti-BDNF antibody (N-20/sc 546, Santa Cruz Biotechnology; diluted 1:600) able to detect all BDNF isoforms (Michalski and Fahnstock, 2003; Peng et al., 2005). Following incubation with anti-rabbit secondary antibody (DakoCytomation, diluted 1:10,000), immunoreactivity was detected by chemiluminescence (Amersham Biosciences). Films were scanned using an Epson Scanner (Epson perfection V500-photo) and densitometry of immunoreactive bands of 14 KDa, 28 KDa and 32 KDa apparent molecular weight was determined by Quantity-One software (BioRad Laboratories). For calibration, each gel was loaded with 200 pg of recombinant human BDNF and only films with comparable intensity of this band were used. To avoid signal saturation, densitometry was performed at 12 bit. The relative amount of single proteic BDNF isoforms was evaluated by densitometrical analysis of the single bands Pro-BDNF, truncated-BDNF e Mature BDNF. The abundance of each BDNF isoform for each subject was expressed as a percentage of this isoform with respect to total BDNF in the same subject, calculated as follows: $[\text{densitometric value of a given BDNF-isoform} / \text{sum of the densitometric values (Pro-BDNF + truncated-BDNF + Mat BDNF)}] \times 100$. We used selected areas of equal surface and quantified the integrated density for each isoforms. We subtracted from each band the specific

background signal collected from each film developed. We verified that all purified sera were loaded on western-blot in comparable amount ($\sim 10\%$) using the PonceauS staining (2% in TCA, SigmaAldrich). However, a normalization for loading was not necessary because we calculated the ratio within-subject.

7.5. Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences, SPSS 15.0 software (SPSS Inc.). The Kolmogorov-Smirnov test was employed to analyse the distribution of populations. Patients and healthy controls' continuous data were compared using the Student's t test and the Mann-Whitney U test, as appropriate. The χ^2 test was used to compare categorical data. Statistical significance was set at 0.05 (two tailed). To quantify statistical differences in serum BDNF levels between patients and healthy donors first, ELISA data on total serum BDNF were evaluated by U-Mann-Whitney test and then, Western-blot data on BDNF isoforms relative abundance were statistically analysed using one-way ANOVA and pairwise multiple comparison procedure. Partial correlation analysis was used to analyse association between clinical and neuropsychological measures controlling for age and length of illness (Statistical significance was set at $p < 0,05$). Multivariate analysis of covariance (MANCOVA) was used to detect differences between groups of patients assuming typicals, atypicals or both in terms of serum pro-BDNF isoforms/total BDNF ratio, with age, gender and duration of therapy as covariates. Spearman's correlations between cognitive data and mature BDNF, truncated-BDNF 28 KDa and precursor of 32 KDa were performed within each group.

7.6. Calculation of test performance

Positive individuals had values $>2D$ or $<2SD$ with respect to the mean value in the normal healthy population. Schizophrenic patients (SZ) positive to test are true positive ($=a$), healthy controls (HC) which tested positive are false positive ($=b$), SZ that tested negative are false negative ($=c$) while HC that tested negative are true negatives ($=d$). Sensitivity, calculated as $a/(a + c)$, measures the proportion of actual positives which are correctly identified as such. Specificity, calculated as $d/(d + b)$ measures the proportion of negatives which are correctly identified. The positive predictive value is the probability a test-positive is a true positive: $a/(a + b)$. The negative predictive value is the probability a test-negative is a true negative: $d/(c + d)$.

8. Results

We studied a group ($n=40$) of chronic SZ patients in comparison with a demographically matched healthy controls group (HC, $n=40$) with comparable male/female ratio (20/20), age, years of education, BMI, and smoking habits. SZ subjects enrolled in the study had evidence of continued illness (mean illness duration 23.05 years \pm 10.99), a score on Positive and Negative Syndrome Scale (PANNS; Kay et al., 1989), and significantly reduced IQ with respect to HC. All SZ patients were under treatment with typical or atypical antipsychotics monotherapy or combined therapy (see Methods for details). Neurocognitive performances, measured using an abbreviated neuropsychological assessment (Harvey et al., 2009), were significantly different between HC and SZ groups ($p < 0.0001$).

	HC subjects (n = 40)	SZ subjects (n = 40)	p value
Gender (Male/Female)	20/20	20/20	1 ^b NS
Age (years)	44.5; 37.25–57.25	48; 42.25–58	0.214 ^a NS
Education (years)	13; 12.25–15.75	13; 10.25–15	0.867 ^a NS
IQ	103.00; 98.00–108.75	98.00; 88.00–100.00	0.0001 ^a
BMI (kg/m ²)	23.55; 21.15–27.5	23.95; 21–28.92	0.637 ^a NS
Smokers	23	30	0.155 ^b NS
Duration of illness (years)	–	23.05 ± 10.99	–
PANSS positive symptoms	–	17.25 ± 7.81	–
PANSS negative symptoms	–	25.27 ± 9.51	–
PANSS general symptoms	–	31.07 ± 6.30	–
PANSS total score	–	73.60 ± 13.00	–
Trail Making Part A time	45.5; 42.25–48	63; 60–69.5	0.0001 ^a
Trail Making Part B time	139.5; 135.25–146	159; 156–160	0.0001 ^a
WAIS-III Digit symbol	55; 53–58	37.5; 37–39	0.0001 ^a
WAIS-III Digit Span Forward	10; 9–12	8; 7–9	0.0001 ^a
WAIS-III Digit Span Backward	7; 6–7.75	5; 4.25–7	0.0001 ^a
Wais-III Letter-Number Sequencing	7; 6–8	5; 5–7	0.0001 ^a
Total serum protein (g/dl)	7.05; 6.7–7.57	6.95; 6.42–7.4	0.231 ^a NS
Platelets (n° 10 ³ /μL)	243; 234–254.75	243; 234–263	0.566 ^a NS
Total serum BDNF (ng/ml)	28.9; 22.0–32.1	25.5; 23.1–27.8	0.018 ^a

Mean ± standard deviation. HC, healthy control; SZ, schizophrenia; PANSS, Positive and Negative Syndrome Scale; BMI, Body Max Index.

NS = non significant.

^a U Mann–Whitney test (Shown is median; Q1–Q3).

^b χ^2 square (Shown mean + Standard deviation).

Table 7. Socio-demographic, clinical and neurocognitive characteristics and serum BDNF levels of the sample.

8.1 Measurement of serum BDNF

As measurement of serum BDNF is influenced by total serum protein concentration and number of platelets (Karege et al., 2005) we measured these serum parameters in HC and SZ and found no differences between the two groups. We also did not find any significant gender difference between BDNF serum values of healthy donors, as reported previously (Ziegenhorn et al., 2007). The SZ group showed a slightly lower mean serum concentration of BDNF measured by ELISA, with respect to the HC group (Table 1, Fig. 1; $p < 0.018$). However, by comparing the distribution of serum concentrations for BDNF in HC and SZ subjects, it was evident that the two populations had an overlapping range of values. These results confirmed that it is practically impossible to discriminate between SZ and HC only on the basis of BDNF serum levels. Hence, we investigated by Western-blot analysis the abundance of the different BDNF isoforms in the serum of these two groups. Recombinant human mat-BDNF and pro-BDNF precursor were loaded as positive controls (first two lanes).

Densitometric analysis of Western blots (n=40 HC, n=40 SZ, each in duplicate), showed that with respect to HC, SZ subjects had significantly more pro-BDNF (SZ 47.31% \pm 9.05, HC 32.72% \pm 9.27; $p < 0.001$), more mat-BDNF (SZ 31.07% \pm 8.69, HC 26.26% \pm 5.69; $p < 0.001$) and reduced truncated-BDNF (SZ 21.62% \pm 10.04, HC 41.01% \pm 8.6; $p < 0.001$). Values are expressed as % of total BDNF, see Methods). For comparison with other studies using antibodies against the proregion common to both pro-BDNF and truncated-BDNF, we also calculated the relative abundance of prototal given by the sum of pro-BDNF and truncated BDNF and found a significant decrement in SZ patients with respect to HC. To analyse individual variability, three scatter plots were generated, for pro-BDNF, truncated BDNF and mat-BDNF, respectively and patients were subdivided in subgroups using as reference the mean value of the HC group and corresponding standard deviation values (SD, Fig. 2C,D and E). Although some SZ patients had BDNF isoforms abundance comparable to those of HC, we found three subgroups of SZ subjects with clearly altered values. The first subgroup, consisting in 15 out of 40 SZ subjects, was characterized by pro-BDNF values greater than the HC mean \pm 2SD. The second subgroup (n = 9/40 SZ subjects) showed mat-BDNF values greater than the HC mean \pm 2SD and the third, more numerous, subgroup (n=27/40) comprised SZ subjects with truncated-BDNF values lower than the HC mean-2SD. Interestingly, each SZ patient with mat-BDNF (Group 1) or pro-BDNF (Group 2) levels greater than the HC mean \pm 2SD ($>2SD$), also had a reduction in truncated-BDNF with values lower than the HC mean-2SD ($<2SD$). However, these two groups (Group 1 and 2) were distinct because we never found a case in which both mat-BDNF and pro BDNF were significantly increased at the same time. Of note, these two subgroups were not significantly related to a particular atypical and/or typical antipsychotic therapy ($\chi^2 = 0.940$, df $\frac{1}{4}$ 2, $p \frac{1}{4}$

0.625). In conclusion, this analysis underlined that every patient with truncated-BDNF levels lower than HC mean-2SD also had significantly altered amount of pro-BDNF or mat-BDNF. Thus, to detect both Group 1 and 2 SZ patients with an unbalance in BDNF isoforms levels, it is sufficient to quantify serum truncated-BDNF.

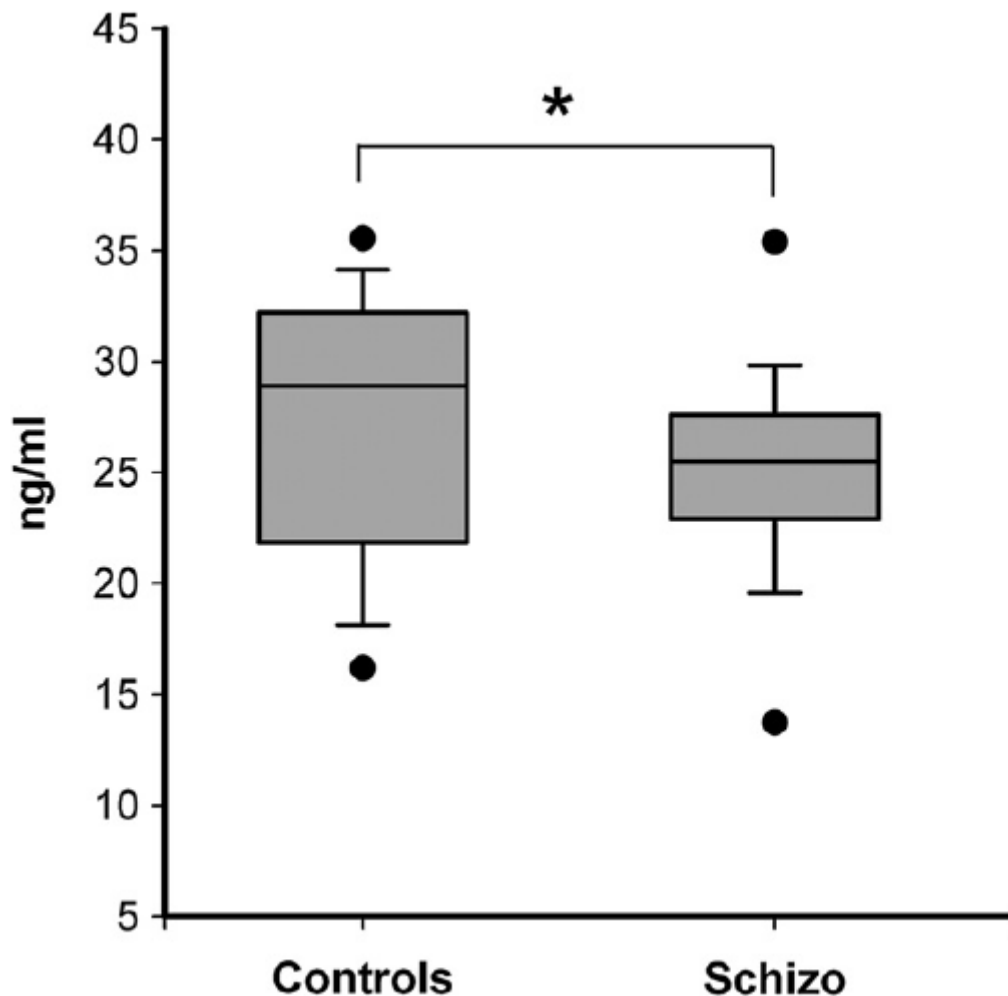


Figure 11. BDNF serum values. Box plot of serum BDNF concentration (pg/ml) in healthy human volunteers (Controls, n = 40) and schizophrenic patients (Schizo, n = 40). The upper line of the box corresponds to the 75th percentile (Q3), the middle line is the median value and the lower line marks the 25th percentile (Q1). HD Controls median = 28.9; Q1-Q3 = 22.0-32.1. Schizophrenic patients median = 25.5; Q1-Q3 = 23.1-27.8. Whiskers (error bars) above and below the box indicate the 90th

and 10th percentiles. Dots indicate the highest and lowest value within each group. * = $p < 0.018$, Mann-Whitney U test.

8.2. Association of truncated-BDNF levels to cognitive impairment

Since not all SZ patients had reduced truncated-BDNF, we investigated if there were differences between SZ with normal truncated-BDNF (13/40) and those with reduced truncated-BDNF (27/40). SZ with low truncated-BDNF had a longer disease duration and significantly higher positive and lower negative PANNS scores, but were not different in PANSS general symptoms and total score with respect to SZ with normal truncated-BDNF. We found that in SZ patients, low truncated-BDNF expression correlates with worse performance on Trail Making Test Part B ($r = 0.553$; $p < 0.0001$), Digit Symbol Coding ($r = 0.360$; $p = 0.046$) and Digit Span Forward ($r = 0.360$; $p < 0.024$) but not Trail Making Test part A. Moreover, within the group with reduced truncated-BDNF, we found a significant correlation between PANSS Negative Symptoms scores and neurocognitive tests scores except for Trail Making Test Part A ($p < 0,05$). Finally, we also examined cognitive functioning of healthy control subjects with low truncated-BDNF and observed reduced performances in three out of four subjects on test of information processing (Trail Making Test B; case 1: 150; case 2: 148; case 3: 150; case 4: 143; see median and 25th and 75th percentiles of HC subjects) and in all four individuals on attention (Symbol Digit Coding; case 1: 50; case 2: 51; case 3: 52; case 4: 52; compare with HC values), even if the low number of cases was insufficient for statistical analysis. The subgroup of SZ patients with elevated pro-BDNF ($>2SD$; Group 1), comprised only subjects who also had reduced truncated-BDNF. Notably, Group 1 SZ patients with elevated pro-BDNF (15/40

subjects) showed the same clinical features of the group with reduced truncated-BDNF. We concluded that augmented pro-BDNF and decreased truncated-BDNF represent two complementary aspects of the same phenotype. However, a subgroup of 9 patients with decreased truncated- BDNF had increased mat-BDNF but normal pro-BDNF (Group 2). Comparative analysis of the clinical features between subgroups revealed that Group 2 patients with increased mat-BDNF, were younger (median 47 years vs. 59) but with significantly longer disease duration (median 31 years vs. 22) with respect to Group 1 patients with augmented pro-BDNF, and were not significantly different with respect to PANSS and cognitive assessment scores. Since antipsychotics can cause a decrease in serum BDNF levels (Jindal et al., 2010), we used multivariate analysis of covariance (MANCOVA) to detect differences between groups of patients assuming typicals, atypicals or both in terms of BDNF isoforms/total BDNF ratio, with age, gender and duration of therapy as covariates. MANCOVA analysis showed that there was no difference between types of antipsychotic medication for the variance of pro-BDNF/tot-BDNF ($F = 0.333$, $df = 2$, $p = 0.719$), truncated-BDNF/tot-BDNF ($F = 1967$, $df = 2$, $p = 0.155$), total pro-BDNF/tot-BDNF ($F = 0.699$, $df = 2$, $p < 0.504$) or mature BDNF/tot-BDNF ($F = 0.699$, $df = 2$, $p < 0.504$). In conclusion, decreased abundance in serum truncated-BDNF BDNF predicts specifically for higher cognitive deficits in schizophrenia and we found a substantial homogeneity in the clinical features of SZ patients with reduced truncated-BDNF, irrespective of whether they also had a concomitant increase in mat-BDNF or pro-BDNF, or antipsychotic therapy used. We evaluated then, if measurement of serum truncated-BDNF could be used as an empirical method to predict for cognitive impairment in both healthy and schizophrenic subjects. This hypothetical test, considering a cut-off at 2SD from the mean value of healthy

controls (i.e., when truncated-BDNF is below 23,79% of the total BDNF serum concentration), has a sensitivity of 67.5% (27/40 SZ are true positive = a, and 13/40 SZ are false negative = c; see Methods), and 97.5% specificity (1/40 HC are false positive = b, thus 39/40 HC are true negative = d; see Methods). Positive Predictive Value is 96.4% and Negative Predictive Value 75% (see Methods). In sum, this test is a very good inclusion test as all subjects with truncated-BDNF below 23,79% are certainly having cognitive impairment.

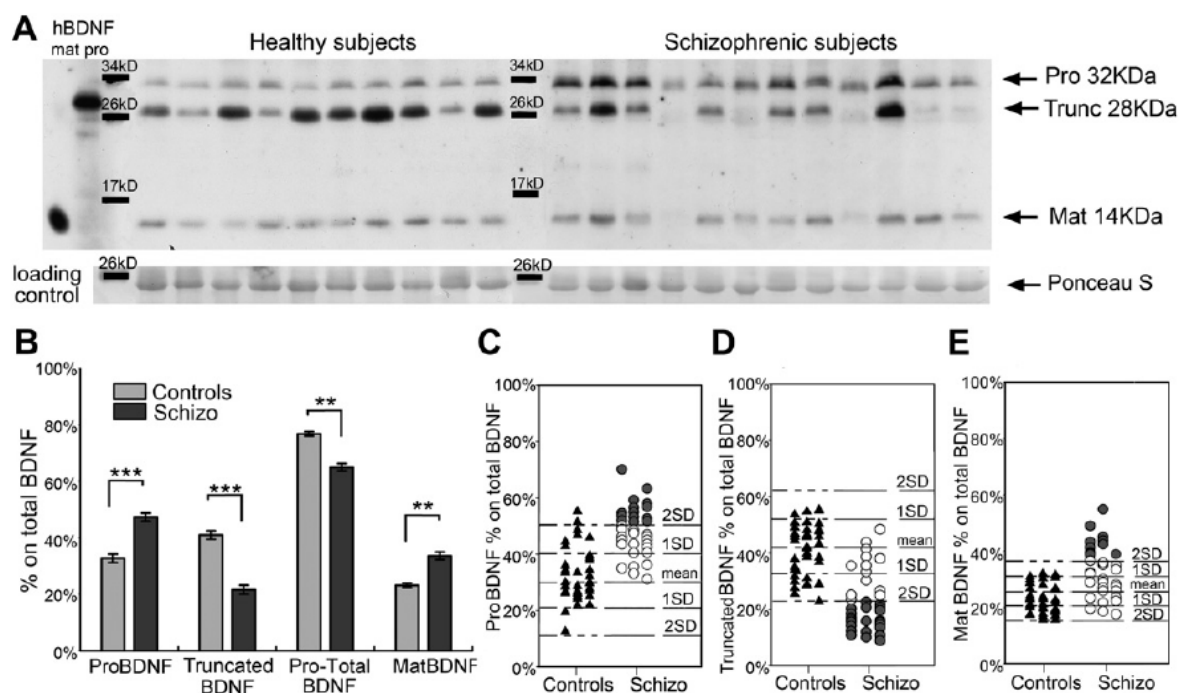


Figure 12. BDNF isoforms in the serum of healthy subjects and chronic patients with schizophrenia. A) Western-blot with sc-546(N-20) anti-BDNF antibody to determine the ratio between pro-BDNF and mature BDNF in healthy controls (HC) and schizophrenic patients (SZ). First two lanes, 500 pg of recombinant human mature BDNF (mat), and 500 pg of recombinant human pro-BDNF (pro). Following lanes, 30 mg of serum proteins of HC or SZ were loaded. Bars with 34 KDa, 26 KDa and 17 KDa indicate position of molecular markers and their relative approximate weight. B) Relative percentage of pro-BDNF, truncated-BDNF or mature BDNF with respect to the total amount of serum BDNF (total BDNF is calculated as the sum of pro-BDNF + truncated-BDNF + mature BDNF densitometric values for each patient). Pro-Total BDNF is the sum of pro- and truncated-BDNF. (CeE)

Scatter plots showing the distribution of the percentage of pro-BDNF (C), truncated-BDNF (D) or mature BDNF (E). Lines within the scatter plot, mark the mean percentage value in the HC group (mean) and the mean plus 1, or 2 standard deviations (1SD, 2SD). * = $p < 0.05$; ** = $p < 0.001$; *** = $p < 0.0001$ one-way ANOVA (HolmeSidak).

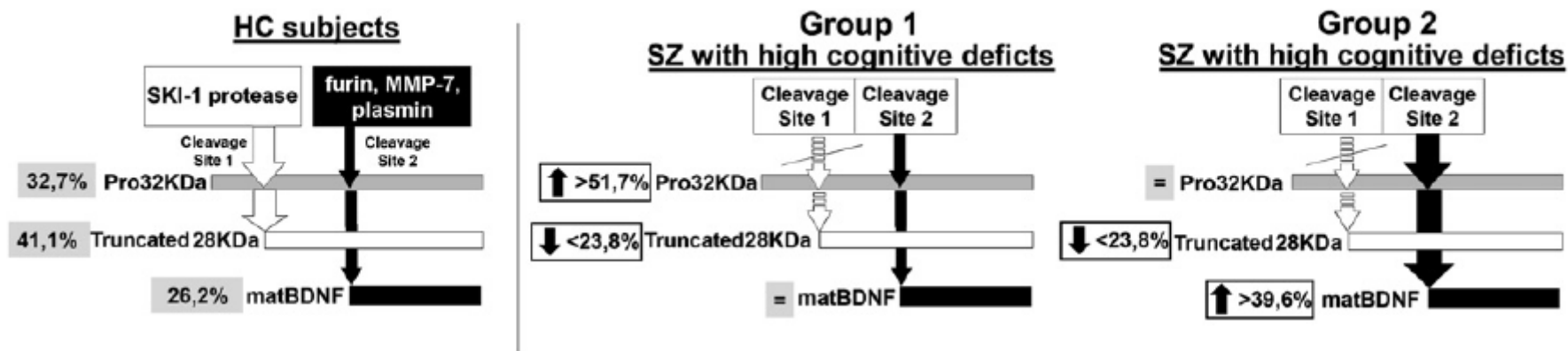


Figure 12. Schematic summary of findings. A) The relative abundance (%) of BDNF isoforms in healthy control (HC) subjects is reported in grey boxes. Cleavage site 1, which generates the truncated-BDNF of 28 KDa form, is cleaved by the SKI-1 protease, while cleavage site 2, which generates mature BDNF, can be recognized by three proteases, furin, MMP-7 and plasmin. B) In Group 1 SZ patients with high cognitive impairment, truncated-BDNF is reduced and uncleaved pro-BDNF precursor is increased, most likely as a result of a reduced cleavage at site 1 while mature BDNF is in normal amounts. In Group 2 SZ patients with high cognitive impairment, truncated-BDNF is also reduced but pro-BDNF is normal (¼no significant variations with respect to healthy controls), most likely due to a compensatory increase of cleavage at site 2 to generate mature BDNF which results increased. As Group 2 patients had longer disease duration with respect to Group 1, this compensatory mechanism may occur after a prolonged pathological condition. Grey boxes report the relative abundance (%) of serum BDNF isoforms which is significantly predictive for high cognitive impairment.

	Normal truncated/ Tot-BDNF	Reduced truncated/ Tot-BDNF (>2 SD = positive)	p value	Normal pro/ Tot-BDNF	Increased pro/Tot-BDNF (>2 SD = positive)	p value	Group 1 increased pro/ Tot-BDNF	Group 2 increased Mat/ Tot-BDNF	p value
Age	45; 36.5–51	50; 46–60	0.013 ^b	46; 40–54	58; 48–62	0.007 ^b	59; 46.5–62	47.5; 39.5–52	0.021 ^b
Duration of illness (years)	16; 8–20	25; 18–35	0.001 ^b	19.74 ± 10.19	28.60 ± 11.35	0.017 ^a	22.00 ± 10.99	31.50 ± 10.86	0.019 ^a
PANSS positive symptoms	18; 16–21	14; 9–20	0.031 ^b	18; 15–21	11; 8–17	0.013 ^b	11; 8–16.75	14; 9–17	0.497 ^b NS
PANSS negative symptoms	23; 21–26	29; 24–35	0.034 ^b	23; 19–29	29; 26–36	0.003 ^b	29; 25.25–36.5	30.5; 27–33.5	0.771 ^b NS
PANSS general symptoms	30.92 ± 4.57	31.15 ± 7.17	0.905 ^a NS	31.04 ± 4.74	30.87 ± 8.60	0.460 ^a NS	26.5; 23.5–40.75	33; 29–38	0.381 ^b NS
PANSS total score	74.38 ± 8.99	70.56 ± 14.68	0.316 ^a NS	72; 63–80	63; 59–93	0.930 ^b NS	63; 59–95	80; 64.5–86.25	0.314 ^b NS
Trail Making Part A Time	61.85 ± 2.23	66.04 ± 8.08	0.017 ^a	61; 59–64	67; 61–74	0.038 ^b	65.2 ± 7.04	69.92 ± 7.29	0.141 ^a NS
Trail Making Part B Time	156.92 ± 2.21	158.77 ± 3.76	0.012 ^b	158; 154–160	159; 159–162	0.003 ^b	159.5; 159–162	160; 159–162	0.923 ^b NS
WAIS-III Digit symbol	156; 155–159	159; 159–162	0.011 ^b	38; 37–39	37; 37–38	0.033 ^b	37; 37–37	37; 36–37.25	0.771 ^b NS
WAIS-III Digit Span Forward	39; 38–39	37; 37–38	0.011 ^b	7; 6–9	6; 5–7	0.038 ^b	7.5; 7–8	7; 7–8	0.582 ^b NS
WAIS-III Digit Span Backward	9; 8–9.5	8; 7–8	0.005 ^b	6; 5–7	5; 4–5	0.004 ^b	5; 4.25–5	4; 4–5	0.107 ^b NS
Wais-III Letter-Number Sequencing	6; 6–7	5; 4–6	0.042 ^b	6; 5–7	5; 4–5	0.002 ^b	4.5; 4–5	5; 4.75–5.25	0.283 ^b NS

NS = non significant.

^a Student's *t* test (shown is mean value ± Standard deviation).

^b Mann–Whitney test (Shown is median; Q1–Q3). Pro32 = pro-BDNF uncleaved precursor (32 KDa); pro28 = truncated pro-BDNF (28 KDa); MAT = mature BDNF(14 KDa); TOT = sum of pro32 + pro28 + mature BDNF.

Table 8. Association of reduced pro28 BDNF with worst PANNS symptoms and greater cognitive impairment.

9. Discussion

In this study we provide evidence that reduced levels of serum truncated-BDNF/total BDNF ratio correlate with worst PANSS negative and positive symptoms and poorer neurocognitive performance. Instead, measurement of total serum BDNF levels resulted poorly informative, even if we found a small decrease in the whole population of schizophrenic patients. We further show that when using a cut-off at mean \pm 2SD from the healthy group, measurement of relative amount of serum truncated-BDNF provides a novel empirical test to identify schizophrenic patients with high cognitive deficits, with sensitivity $\frac{1}{4}$ 67.5%, Specificity $\frac{1}{4}$ 97.5%, PPV $\frac{1}{4}$ 96.4% and NPV $\frac{1}{4}$ 75%. Regulated proteolysis of one inactive precursor to produce active peptides and proteins is a general mechanism to generate biologically diverse products from a single gene. Mammalian pro BDNF precursor is processed to generate truncated-BDNF 28 kDa or mature 14 kDa BDNF by two different proteolytic cleavages. Mature BDNF is generated intracellularly by furin (Mowla et al., 2001), or extracellularly, by plasmin or matrixmetalloprotease-7 (Lee et al., 2001). Truncated-BDNF is generated by a specific Ca^{2+} -dependent serine proteinase known as Membrane-bound transcription factor site-1 protease (MBTFS-1), also known as Subtilisin/kexin-isozyme 1 (SKI-1) (Seidah et al., 1999) and since is not further processed into the mature 14 kDa BDNF form, it represents a true final proteolytic product whose function is unclear. Recent studies have demonstrated that mature and pro-BDNF elicit opposite biological effects (Teng et al., 2005; Woo et al., 2005), leading to the conclusion that an incorrect balancing of the different isoforms may cause a pathological effect.

More recently, Koshimizu et al. (2009) reported that overexpression of pro-BDNF elicited apoptosis of cultured cerebellar granule neurons and caused a dramatic reduction in the number of cholinergic fibers of basal forebrain neurons and hippocampal dendritic spines, without affecting the survival of these neurons. Blockade of activation of p75 receptor prevented spine number reduction. Of note, the pro-BDNF preparation used by these authors consistently contained truncated BDNF, albeit at a much lesser extent than pro-BDNF. It is therefore conceivable that truncated-BDNF may have a similar effect than pro-BDNF through formation of homodimers. As a possible alternative, truncated-BDNF may be an inactive form of pro-BDNF or act as a quencher of pro-BDNF by forming inactive heterodimers. The latter hypothesis recalls the proposed role for truncated-TrkB. Hence, a marked reduction in truncated-BDNF may lead to pathological increased signalling of pro-BDNF. Here, we provide evidence that 2/3 of schizophrenic patients present an altered regulation of the 28 KDa BDNF truncated proteolytic product. Further studies will be required to elucidate the biological role of truncated-BDNF. We must emphasize that all patients with reduced truncated-BDNF BDNF showed either increased pro-BDNF (Group 1) or increased mat BDNF (Group 2) but not both. Fig. 12 shows a possible model to explain how this dysregulation might occur. In Group 1 SZ patients, reduced cleavage at site 1 produces less truncated-BDNF and results in accumulation of uncleaved pro-BDNF precursor while cleavage at site 2 is unaffected and therefore, mature BDNF levels are normal. In Group 2 SZ patients, even if truncated-BDNF is reduced like in Group 1 patients, the increase in pro-BDNF does not occur, most likely due to a compensatory increase in processing of this precursor to generate the mature 14 KDa form. Indeed, in Group 2 SZ patients we observed an increase in mature BDNF. Considering that this increase is noticed in Group 2 SZ patients with

longer disease duration (median 31.5 vs. 22 years in Group 1), it is possible to hypothesize that this compensatory mechanism may occur after a prolonged pathological condition, and does not operate in Group 1 SZ patients. These observations warrant further investigations to determine whether the unbalance in serum BDNF isoforms is due to altered expression or efficiency of proteases, serum protease inhibitors levels or mutations at BDNF cleavage sites. Chronic schizophrenic patients are consistently found to undergo progressive substantial intellectual deterioration at below average levels of functioning (Heinrichs, 2006). In our study, SZ patients with reduced truncated-BDNF had worse performance in all neurocognitive tests with respect to the other patients with normal levels of truncated-BDNF, although no significant correlation was found between Trail Making Test Part A score and truncated-BDNF abundance in patients with schizophrenia. Our results further extend a recent study suggesting that measurement of total serum BDNF may be of use to monitor for a successful training for cognitive enhancement in schizophrenic patients (Vinogradov et al., 2009). Of note, we also found that four healthy controls with low truncated-BDNF had poor scores in Trail Making Test B and Symbol Digit Coding attention test. On the basis of our findings it is expected that SZ patients with low serum truncated-BDNF (and worse cognitive impairment) are likely to be more refractory to a non-pharmacological cognitive rehabilitation therapy. We used six neuropsychological tests that are consistently correlated with functional outcomes. In particular, four out of these six measures explained 74% of variance in the composite score analysis (Harvey et al., 2009). Notably, we found in SZ patients with reduced truncated-BDNF a significant association between higher PANSS Negative Symptoms scores and worse neurocognitive tests scores except in Trail Making Test Part A. A recent meta-analysis (Dickinson et al., 2007) reported that

reduced processing speed performance at the digit symbol coding task represents the greatest deficit among cognitive abilities in patients with schizophrenia and there is cleavage at site 1 while mature BDNF is in normal amounts. In Group 2 SZ patients with high cognitive impairment, truncated-BDNF is also reduced but pro-BDNF is normal (¼no significant variations with respect to healthy controls), most likely due to a compensatory increase of cleavage at site 2 to generate mature BDNF which results increased. As Group 2 patients had longer disease duration with respect to Group 1, this compensatory mechanism may occur after a prolonged pathological condition. Grey boxes report the relative abundance (%) of serum BDNF isoforms which is significantly predictive for high cognitive impairment.

10. Future research proposals

The hypothesis that relapse could be predicted by low neurotrophin levels is consistent with the neurobiology of relapse and with preliminary data in first episode psychosis patients (Parikh et al., 2003). Therefore, large populations of high-risk subjects or untreated first episode patients need to be longitudinally investigated to improve the statistical importance of the analysis (Pantelis et al., 2003). In fact, in the absence of a neuroleptic naïve cohort followed longitudinally to evaluate pattern of neurotrophins over time, it is difficult to determine whether any relationship between relapse and low neurotrophins would be due to an underlying neurobiological vulnerability to relapse, an inadequate therapeutic response to antipsychotics, or inadequate antipsychotic exposure due to medication noncompliance. In the first part of the meta-analysis, serum BDNF levels were shown to be reduced in patients with schizophrenia even if the difference was moderately significant ($p < 0.05$). However,

considerable statistical heterogeneity was detected between studies. In the second part of the meta-analysis, we found that serum BDNF levels in patients with drug free/first episode psychosis were significantly lower in patients compared to healthy control subjects, but we could not detect any significant alteration in serum BDNF levels in patients with chronic schizophrenia. In both cases, a high heterogeneity was between the studies was highlighted and it is still unclear whether the reduction in serum BDNF levels observed in drug naïve/first episode patients with schizophrenia is due more to antipsychotic treatment or toxic effect of psychosis in itself. Therefore, future biochemical studies should longitudinally investigate larger samples of high-risk individuals, drug free first-episode patients and unaffected family members. Such populations are crucial to systematically examine whether serum BDNF levels changes are already present before the appearance of symptoms, or whether they develop afterwards, as a result of the course of illness. Such biochemical studies, should be crossed with MRI, genetic and metabolism investigations data, in order to further investigate whether serum BDNF levels represent an indicator of vulnerability to the disease and to better understand the functional expression of serum BDNF levels abnormalities in schizophrenia.

11. Conclusions

Taken together, these findings suggest that the processing of precursors of BDNF may be a key regulator of synaptic plasticity which is the neurobiological underpinning of brain remodeling and cognitive functioning. The preclinical studies reviewed here pointed to the role of aging on BDNF proteolytic processing, which may have a significant impact on other biological cascades (i.e. Beta-amyloid or LPS-mediated inflammatory processes). Changes in BDNF catabolism may have an additive and/or multiplicative neuropathological effect on brain's structure, thus perpetuating neurodegenerative events in schizophrenia as well as AD. Indeed, neuroimaging studies showed volumetric reduction at the level of the hippocampus and frontal lobe in schizophrenia, and similar findings were observed in AD. In AD, serum BDNF changes may also indicate a progressive shift toward a neurodegenerative state in the healthy elderly subjects and progression from aMCI to AD. It is plausible to hypothesize that, in complex neurodegenerative diseases, protective and deleterious biochemical cascades are in a delicate homeostatic balance that is tightly controlled and, thus, neurodegeneration results from an imbalance between these cascades. Respect to schizophrenia, convergent evidence of age-related decline in blood and brain BDNF might therefore reflect an accelerated decline in normal age-related BDNF decrease. In addition, a great number of studies sustain that a significant reduction of peripheral and CNS BDNF levels occurs early in the development of schizophrenia brains. Moreover, this reduction is more consistent than that observed in healthy subjects during aging. Modern pathophysiological hypotheses of schizophrenia postulate both neurodegenerative and neurodevelopmental processes modify brain structure and participate in the

development of clinical symptomatology. To date, there are several brain tissue morphological alterations deriving from abnormal neural organization (i.e. esempi con riferimenti letterari) in schizophrenia. However some regions may face a further degeneration, as the dopaminergic pathways directed to the cortex and glutamatergic pathways directed from the cortex to subcortical structures. This neurobiological events may explain the progressive resistance to antipsychotic medications and worsening of cognitive impairment. Degeneration may be also encouraged by positive symptoms in psychotic relapse, as they have a neurotoxic impact. Expanding the Buckley point of view (Buckley, 2007), BDNF metabolism is both a 'biological footprint' of relapse in schizophrenia and a plausible endophenotype of "cognitive stress": a strain in cognitive function or processing certain information/messages sent to the brain. Although the role of different BDNF isoforms remains to be elucidated, we ,we may speculate that truncated-BDNF have a similar outcome than pro-BDNF through activation of the same signaling pathways. Alternatively, truncated-BDNF may be an inactive variety of pro-BDNF or operate as a quencher of pro-BDNF by producing inactive heterodimers.. Hence, a clear decrease in truncated-BDNF may direct to pathologically amplified signaling of pro-BDNF. Further studies will be necessary to clarify the biological characteristics of truncated-BDNF. Therefore, the activity of proteases mediating BDNF processing may be considered a compensatory neurotrophic mechanism, partly explaining etiopathogenesis, early diagnosis, treatment and prognosis of schizophrenia and AD.

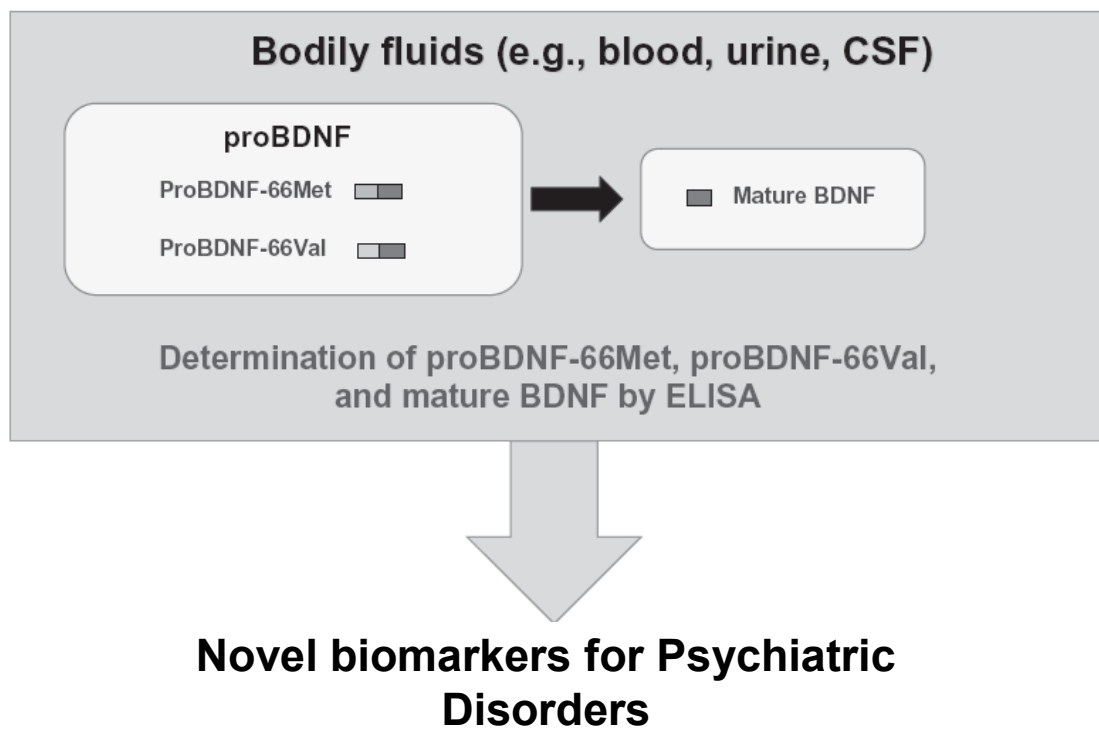


Figure 13.

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