UNIVERSIDADE CATÓLICA DE PELOTAS
PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE E COMPORTAMENTO
LABORATÓRIO DE NEUROCIÊNCIAS CLÍNICAS

ESTUDO DE ASSOCIAÇÃO ENTRE O POLIMORFISMO VAL66MET NO GENE DO BDNF COM TRANSTORNO DE ANSIEDADE GENERALIZADA

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Projeto de pesquisa elaborado para o Mestrado em Saúde e Comportamento da Universidade Católica de Pelotas, sob a orientação da Prof. Dra. Gabriele Ghisleni

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IDENTIFICAÇÃO

Título: Estudo de associação entre o polimorfismo Val66Met no gene do BDNF com Transtorno de Ansiedade Generalizada

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1. Introdução

O transtorno de ansiedade generalizada (TAG) é uma doença crônica, caracterizada pelo excesso de ansiedade e preocupação somada à presença de outros sintomas, como tensão muscular, e irritabilidade (Hanrahan et al, 2013; Zargar et al, 2013). De acordo com o Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-IV), esse transtorno normalmente inicia na adolescência e afeta cerca de 5,7% da população em geral com altas taxas de incidência em mulheres (Kessler et al, 2005; Tempesta et al, 2013). Além disso, o TAG tem considerável impacto na qualidade de vida, sendo responsável por prejuízos na vida social, profissional e familiar dos seus portadores (Zargar et al, 2013; Tempesta et al, 2013). O TAG, geralmente é acompanhado de comorbidades psiquiátricas como a depressão maior, outros transtornos de ansiedade, e abuso de álcool, sendo caracterizado por altas taxas de falha na terapêutica, o que dificulta a resposta ao tratamento, bem como a resposta a psicoterapia (Zargar et al, 2013; Grant et al, 2005).

O TAG é uma doença complexa na qual ocorre a interação de múltiplos fatores ambientais, biológicos e genéticos, cuja neurobilogia exata permanece ainda desconhecida (Zargar et al, 2013; Chen et al, 2006). O fator neurotrófico derivado do cérebro (BDNF), membro da família das neurotrofinas, age sobre certos neurônios do SNC e periférico, desempenhando um papel importante na sobrevivência, diferenciação e crescimento neuronal durante o desenvolvimento e na idade adulta (Gratacos et al, 2007; Egan et al, 2004). No cérebro está presente em regiões específicas como hipocampo, neocortex e hipotálamo, regiões-chave na regulação do humor e comportamento, bem como na aprendizagem e memória, indicando um envolvimento direto na patofisiologia das doenças psiquiátricas (Yoshii and Constantine-Paton, 2010; Lu et al, 2008).

Estudos clínicos e pré-clínicos com transtornos de ansiedade demonstram que alterações no gene e nos níves de BDNF podem estar associados a doença (Hartmann et al., 2001; Rasmusson et al., 2002; Molle et al., 2012). Porém os estudos nesta área são escassos e inconsistentes, mas indicam que indivíduos com TAG apresentam uma maior frequência do alelo Met (Suliman et al, 2013; Ball et al, 2013). Dessa forma, uma melhor compreensão de alguns fatores genéticos envolvidos nos transtornos de ansiedade poderá permitir avanços não só ao nível de tratamento, mas também de

prevenção e diagnóstico, podendo algumas alterações neurobiológicas vir a integrar os critérios de diagnóstico e monitoramento da doença, hoje exclusivamente semiológicos, e contribuir, assim, para a explicação da heterogeneidade desta patologia.

2. Estratégias de Busca

As buscas foram realizadas principalmente na base de dados Pubmed, complementadas com referências dos artigos encontrados, de cujo conteúdo utlizaram-se 12 estudos. A estratégia de busca está descrita na tabela abaixo.

Tabela 1. Estratégia de revisão bibliográfica

Base de Dados	Descritores	Artigos Encontrados	Incluídos no Estudo
PubMed	Val66met and (anxiety or GAD)	74	10
PubMed	rs6265 and (anxiety or GAD)	13	4
PubMed	(rs6265 or Val66Met) BDNF levels	120	15
PubMed	Anxiety Disorder and BDNF	354	8
PubMed	Generalized Anxiety Disorder and BDNF	10	6
Artigos encontrados nas referências de estudos		38	7

3. Fundamentação Teórica

3.1. Transtornos de Ansiedade

Os transtornos de ansiedade são caracterizados como estados emocionais repetitivos ou persistentes nos quais a ansiedade patológica desempenha papel fundamental (Hetem e Graeff, 2004). Além disso, estão associados a um intenso grau de incapacidade e baixo nível de satisfação e qualidade de vida (Ball et al., 2013; Tempesta et al, 2013).

O estudo realizado pelo National Comorbidity Survey (NCS), com diagnóstico baseado na CID-10, confirmou uma prevalência ao longo da vida de 24,9% para os transtornos ansiosos, incluindo fobias específicas da infância e adolescência (Kessler et al.,2000). No Brasil, a prevalência dos transtornos de ansiedade em serviços primários de saúde está entre 26,7% e 39,6% do total dos pacientes atendidos e é duas vezes mais comum em mulheres (Bernik, 2001).

De acordo com o DSM-IV, os transtornos de ansiedade são classificados em TAG, transtorno de pânico com e sem agorafobia, fobia específica, fobia social, transtorno obsessivo compulsivo (TOC), transtorno do estresse pós-traumático (TEPT). Em relação a diagnósticos específicos, o transtorno de ansiedade mais comum é o TAG, o qual normalmente inicia na adolescência e afeta cerca de 5,7% da população mundial (Tempesta et al, 2013; Hetem e Graeff, 2004). O TAG é caracterizado por preocupação e ansiedade excessiva que ocorre na maior parte dos dias por pelo menos 6 meses. Além disso, está associado a manifestação de sintomas somáticos, com múltiplas queixas físicas como fadiga, irritabilidade, tensão muscular e dificuldade de concentração (DSM-IV).

Comorbidades psiquiátricas são comuns em indivíduos com TAG. Aproximadamente 29-62% dos indivíduos apresentam depressão maior e 38% abuso de álcool (38%), dificultando o diagnóstico específico e prejudicando o tratamento (Witchen et al, 2002), além de gerar um intenso grau de incapacidade e um impacto negativo na qualidade de vida (Kessler et al, 2000).

3.2 Fator neurotrófico derivado do cérebro (BDNF)

O BDNF é uma neurotrofina importante na regulação de diversos aspectos do desenvolvimento e funções neuronais incluindo sobrevivência, neurogênese e plasticidade sináptica (Keller et al., 2010). O BDNF está amplamente distribuído no

SNC em regiões importantes para os processos envolvidos na aprendizagem, memória e padrões comportamentais (Gratacos et al, 2010; Pregelj et al, 2011). Alterações na expressão do BDNF podem reduzir a plasticidade neuronal e, portanto, dificultar a adaptação a eventos estressores, contribuindo para o desenvolvimento de doenças neurológicas, como o transtorno bipolar, epilepsia, depressão e suicídio (Egan et al, 2004). Essas modificações podem ocorrer por uma série de respostas como os níveis de glicocorticoides, esteroides sexuais e exercícios físicos (Lou et al, 2008).

Estudos atuais demonstram que os níveis de BDNF no soro são significativamente menores nos indivíduos com TEPT e TOC quando comparados com controles saudáveis (Dell'Osso et al, 2011; Dos Santos et al, 2011). Por outro lado, Hauck e colaboradores (2010), encontraram níveis aumentados de BDNF em pacientes com trauma recente (menos de um ano desde o evento traumático).

Estudos recentes têm demonstrado ainda que o polimorfismo rs6265 no gene do BDNF, o qual decorre da substituição de uma base guanina por uma adenina na posição 196, levando a uma troca do aminoácido valina por uma metionina no códon 66 (Val66Met), pode estar associado à maior susceptibilidade aos distúrbios psiquiátricos (Gratacos et al, 2010; Tempesta et al, 2013). A presença do polimorfismo promove uma alteração funcional em que há uma diminuição do tráfego intracelular e diminuição da secreção de BDNF (Egan et al., 2003; Miyajima et al. 2008), conferindo uma redução no dos níveis no sistema límbico (Chen et al., 2006; Gat et al. 2009.). Essas alterações tem sido relatas em estudos de neuroimagem envolvendo indivíduos com doenças psiquiátricas (Karamohamed et al., 2005; Nagata et al., 2012; Chi et al, 2010; Gruber et al., 2010; Chen et al, 2006; Sun et al., 2012; Skibinska et al., 2004).

Este polimorfismo foi associado com transtorno bipolar, transtornos alimentares, transtorno ao uso de substancias e esquizofrenia (Gratacos et al, 2010), porém sem resultados consistentes em relação a comportamentos ansiosos. Estudos pré-clínicos indicam que esse polimorfismo pode estar implicado no comportamento ansioso. Chen et al, 2006 gerou uma variante do polimorfismo no gene do BDNF em ratos, encontrando que portadores Met/Met tiveram aumento no comportamento relacionado a ansiedade, sustentando a hipótese de que o BDNF tem características neurobiológicas que o tornam um gene candidato de risco para a regulação da ansiedade. Estudos em humanos são escassos, mas sugerem que o alelo Met pode ser um fator de risco para o desenvolvimento de transtornos de ansiedade (Jiang et al, 2005, Hemmings et al, 2008).

4. Objetivos

4.1. Objetivo Geral

Estudar a associação entre o polimorfismo rs6265 (Val66Met) no gene que codifica o BDNF com o diagnóstico de TAG.

4.2. Objetivos Específicos

- Caracterizar a amostra populacional em estudo quanto às características sócio-demográficas e a distribuição dos genótipos.

- Verificar a associação do polimorfismo Val66Met com transtorno de ansiedade generalizada

5. Hipótese

- Ausência de associação entre o polimorfismo Val66Met com características sócio-demográficas.

- Encontraremos uma associação positiva entre o polimorfismo Val66Met no gene do BDNF com transtorno de ansiedade generalizada, na qual indivíduos com TAG apresentarão maior frequência do alelo A em relação aos controles.

6. Metodologia:

6.1. Delineamento do estudo

O estudo segue um delineamento do tipo transversal de base populacional da zona urbana de Pelotas-RS, e faz parte de um estudo maior em andamento intitulado "Estudo do temperamento e transtornos psiquiátricos na interface entre psiquiatria, psicologia e neurociências", aprovado pelo Comitê de Ética da Universidade Católica de Pelotas, protocolo 2010/15.

6.2. Amostra

A seleção amostral encontra-se em andamento sendo realizada por conglomerados, considerando a população de aproximadamente 97 mil adultos de 18 a 35 anos de idade e a divisão censitária atual de 448 setores na cidade de Pelotas-RS, ambos fornecidos pelo Instituto Brasileiro de Geografia e Estatística (IBGE). O tamanho amostral com parâmetros de confiabilidade de 95%, poder de 80%, prevalência do desfecho de 10% e menor prevalência esperada de 8% é de 1714 adultos, a serem avaliados em 68 setores censitários. O cálculo do tamanho amostral realizado com o objetivo de determinar o tamanho mínimo necessário para que seja detectada uma diferença entre os portadores das diferentes variantes genéticas que serão analisadas foi realizado pelo programa PEPI Win, utilizando um intervalo de confiança de 95% e tomando como base o alelo de menor frequência do polimorfismo rs6265 (18%) na população europeia visando encontrar uma diferença significativa (α=0.05). Baseado no tamanho amostral de 1714, a amostra inicial será de 201 controles e 201 pacientes com depressão. Os critérios de inclusão adotados serão: 1) ter entre 18 e 35 anos de idade; 2) residir na zona urbana de Pelotas-RS; bem como no domicílio sorteado; 3) aceitar participar e assinar o termo de consentimento livre e esclarecido. Quanto aos critérios de exclusão consta a incapacidade dos indivíduos em responderem à entrevista por problemas físicos ou cognitivos.

6.3. Instrumentos de avaliação e coleta de dados

As características da amostra serão determinadas através do questionário sócio-demográfico, incluindo avaliação socioeconômica. Os transtornos psiquiátricos de Eixo I do DSM-IV serão avaliados através do Mini Internacional Neuropsychiatric Interview (MINI), para confirmação do diagnóstico de depressão.

A coleta do material biológico será realizada pela equipe de bioquímicos e enfermeiros através de punção venosa no momento da aplicação do questionário, no qual entrevistadores treinados (psicólogos ou psiquiatras) aplicam os instrumentos e avaliação diagnóstica.

6.4. Aspectos éticos

Serão respeitados todos os princípios éticos estabelecidos pelo Conselho Nacional de Saúde na Resolução Nº 196 de 10 de Outubro de 1996. Será assegurado o direito à confidencialidade dos dados e o cuidado na utilização das informações nos trabalhos escritos, de modo que os participantes não possam ser identificados. As pessoas que apresentarem transtornos psiquiátricos receberão encaminhamento para atendimento psicológico/psiquiátrico no Campus da Saúde da UCPel.

6.5. Análise do polimorfismo

O DNA total será extraído a partir de leucócitos do sangue periférico utilizando-se o método descrito por Lahiri e Nurnberger (1987). Após a extração, o DNA total será quantificado por espectrofotometria e armazenado a -20°C até a análise molecular. O polimorfismo rs6265 (Val66Met) será genotipado utilizando-se ensaios de discriminação alélica por PCR em tempo real no termociclador *7500 Fast Real-Time PCR System* (Applied Biosystems, Foster City, CA, EUA). *Primers* e sondas do tipo *TaqMan MGB* serão designados utilizando-se o software *Primer Express v3.0* e a seqüência consenso do gene obtida a partir do *GeneBank* (HTTP://www.ncbi.nlm.nih). As reações de PCR serão realizadas utilizando-se o tampão *TaqMan Genotyping Master Mix* (Applied Biosystems), 900nmol/l de cada *primer*, 200nmol/l cada sonda (VIC e FAM) e 2ng de DNA, conforme determinações do fabricante. Os resultados serão analisados no software *System Sequence Detection* v.1.4 (Applied Biosystems).

6.6. Análise estatística

Os questionários serão digitados e codificados diretamente no programa EpiInfo6, no momento da entrevista, sem necessidade de usar questionário de papel. As frequências genotípicas e alélicas serão estimadas por contagem direta dos alelos e o equilíbrio de Hardy-Weinberg, será testado pelo teste de qui-quadrado. As distribuições alélicas e genotípicas entre os grupos de indivíduos serão avaliadas pelo qui-quadrado. Uma medida de magnitude de efeito será calculada através da razão de chances (odds ratio – OR) e intervalo de confiança de 95%. Para a comparação de variáveis continuas com distribuição normal entre os grupos de pacientes serão utilizados o testet *t de*

Student ou análise da variância (ANOVA), e o teste do qui-quadrado para comparação entre variáveis nominais. Valores de $p \leq 0,05$ serão considerados estatisticamente significativos. As análises estatísticas serão realizadas através do pacote estatístico SPSS 16.0 para Windows.

7. CRONOGRAMA DE ATIVIDADES

Atividade	1º Semestre 2012	2º Semestre 2012	1º Semestre 2013	2º Semestre 2013
Revisão Bibliográfica				
Elaboração do Projeto				
Análise do Polimorfismo (PCR)				
Coleta dos dados				
Análise dos dados				
Elaboração do Artigo				
Apresentação do artigo				

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ARTIGO 1

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Val66Met polymorphism is associated with increased BDNF levels in generalized anxiety disorder.

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Running title: Val66Met polymorphism in anxiety disorder

Key words: Anxiety disorders, generalized anxiety disorder, brain-derived neurotrophic factor, Val66Met polymorphism, neurotrophin.

Abstract

Background: Generalized anxiety disorder (GAD) is a common psychiatric disorder characterized by constant worry or anxiety over everyday life activities and events. The neurobiology of the disorder is until now unclear, although it has been consistently demonstrated that the environment and the genetic profile could increase the risk. We examined whether the BDNF gene, which plays a role in neuroplasticity and memory, could increase the vulnerability to disorder.

Methods: In our study, 723 subjects from a population based study were genotyped by qPCR for the BDNF functional variant rs6265 (Val66Met) and the BDNF serum levels were measured by Elisa.

Results: Our results reveals significant association between the Met66 allele and risk for GAD (p=0.029), but no differences were observed in the serum levels of BDNF according to disease (p=0.652) and the genotype distribution (p=0.222). Furthermore, we show that subjects with GAD carrying the Met66 allele have increased BDNF levels (8.19±2.35 ng/ml) in relation to Val/Val genotype (6.91±2.62 ng/ml; p=0.038), but no association was demonstrated in the control group (p=0.862).

Conclusion: These results suggest that BDNF could be involved in the neurobiology of GAD and might represent a useful marker associated with the disease.

1. Introduction

Generalized anxiety disorder (GAD) is a chronic anxiety disorder characterized by uncontrollable worrying and somatic anxiety, evidenced by increased tension, insomnia and hypervigilance[1,2]. According to *Diagnostic and Statistical Manual IV* (DSM-IV)[3], GAD usually begins in the adolescence and affects about 5.7% of the general population across the lifespan, with the highest incidence among women[4]. Intrinsically linked to stress response, GAD is frequently comorbid with major depression, other anxiety disorders, as well as chronic medical conditions[5,6].

The cognitive and behavioral approach has been widely used during the last two decades to determine the etiology of GAD. However, the neurobiology of the disease remains unclear[2,7]. Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family involved in several forms of plasticity in the nervous system, including neuronal maturation, synaptic remodeling, and long-term memory formation[8,9]. The association between BDNF and the pathophysiology of psychiatric disorders is supported by the inverse correlation found between stress and the levels of this neurotrophin in limbic regions, which re restored after antidepressant treatment[10,11]. In fact, BDNF is involved in anxiety-like behaviors in preclinical models, and numerous types of stressors reduced BDNF expression [10,12,13,14].

A single nucleotide polymorphism (SNP) Val66Met (rs6265) in the coding region of BDNF gene, results from a valine to methionine change at position 66[15,16]. The polymorphism is associated with a functional alteration in which decrease in intracellular trafficking and activity-dependent secretion of BDNF[17,28]. In addition, the Met66 allele of this SNP has been associated with volume reductions in brain areas such as hippocampus, prefrontal cortex, and amygdala[16,19,20] .

The BDNF polymorphism has been associated with several neuropsychiatric disorders, with Met66 allele increasing susceptibility to psychiatric conditions including, GAD [7,21,22,23,24,25,26]. On the other hand, a meta-analysis failed find significant association between the Val66Met SNP and anxiety disorder[27]. Thus, epidemiological studies attempting to link the Val66Met SNP with affective/anxiety disorders have inconsistent results[27,28,29,30]. Although neurobiological mechanisms linking GAD and BDNF are scarcely studied it is of great relevance to clarify the impact of the genotype and peripheral levels of BDNF in the diagnosis and treatment of anxiety disorders. In the present study, we evaluate the role of the functional Val66Met SNP, serum BDNF levels and the vulnerability to GAD in a population-based study.

2. Methods

2.1. Subjects

This cross-sectional study was carried out in 723 subjects participating in a population-based study with people between 18–35 years old, living in Southern Brazil in the period of June 2011 to May 2013. A standard questionnaire was used to collect socio-demographic information, life style, alcohol and tobacco use, psychiatric medication and comorbidities. Ethinicity and the use of psychiatry medication were self-reported in our sample. It is important to highlight that our sample consisted of young subjects possibly experiencing early stages of the illnesses and with a very low use of psychiatric medication. All subjects were evaluated with a structured diagnostic interview—Mini International Neuropsychiatric Interview- MINI[31], that uses the DSM-

IV[3] as criterion for psychiatric diagnostic. The study was approved by the University's Ethics Committee (2010/15) and all patients provided informed consent in writing.

2.2. Biochemical assay

Blood samples were obtained by venipuncture immediately after the interview and clinical diagnostic. The blood was immediately centrifuged at 3,500 x g for 15 min and serum isolated was kept frozen at −80°C. Serum levels of BDNF were measured using a BDNF immunoassay kit (DuoSet ELISA Development, R&D Systems, Inc., USA). Serum BDNF levels are expressed in ng/mL. All samples and standards were measured in duplicates and the coefficient of variation was less than 5%.

2.3. Molecular analyses

DNA was extracted from peripheral blood leucocytes by a standardized salting-out procedure[32]. Genotyping of the Val66Met polymorphism (rs6265) of the *BDNF* gene was determined using forward (GGCTTGACATCATTGGCTGAC) and reverse (GGTCCTCATCCAACAGCTCTT) primers and probes contained in the Human Custom TaqMan Genotyping Assay 40x (Applied Biosystems, Foster City, CA, USA). One allelic probe was labeled with VIC dye and the other was labeled with FAM dye. The reactions were conducted in a 96-well plate, in a total 5 μl reaction volume using 2 ng of genomic DNA, TaqMan Genotyping Master Mix 1x (Applied Biosystems), and Custom TaqMan Genotyping Assay 1x. The plates were then positioned in a real-time PCR thermal cycler (7500 Fast Real PCR System; Applied Biosystems) and heated for 10 min at 95 ºC, followed by 45 cycles of 95 ºC for 15 s and 60 ºC for 1 min. Fluorescence data files from each plate were analyzed using automated allele-calling software (SDS 2..1; Applied Biosystems).

2.4 Statistical analyses

Allelic frequencies were determined by gene counting and departures from the Hardy-Weinberg equilibrium were verified using χ^2-tests. Comparisons of allelic and genotypic frequencies among groups of patients were evaluated using χ^2-tests. Socio-demographic characteristics according to clinical diagnostic and genotypes were compared by using unpaired Student's t-test or χ^2, as appropriate. Variables with normal distribution are presented as mean ± standard deviation (SD) or percentage. Serum BDNF levels with skewed distribution were logarithmically transformed before analyses and are presented as mean ± standard deviation (SD).

3. Results

A total of 723 subjects were included in this report with 620 controls and 103 subjects diagnosed with GAD. Table 1 shows socio-demographic and clinical characteristics of the subjects according to the diagnostic. Our results reveals no differences between control and GAD groups for ethnicity (p=0.224), alcohol use (p=0.184), age (p=0.135), body mass index (BMI) (p=0.095) and serum BDNF levels (p=0.652). However, GAD was more prevalent in women (p=0.001), in subjects with lower socioeconomic class (p=0.006), and higher tobacco use (p=0.001). Although the population of this study has lower use of psychiatric medication (6.5%), the use was higher between GAD subjects when compared to control (p=0.001), but no association for serum BDNF levels was found between medicated (7.2±2.5 ng/mL) and non-medicated subjects (7.4±2.3 ng/mL, respectively; p=0.674).

In the sample, BDNF Val66Met genotype distribution was 72.1% (n=521) homozygous Val/Val, 25.6% (n=185) heterozygous Val/Met, and 2.4% (n=17) homozygous Met/Met. Genotypic distributions of Val66Met SPN did not differ in the GAD diagnostic group (p=0.077), and no allelic difference (p=0.105) was demonstrated. The genotype frequencies were in agreement with those predicted by the Hardy-Weinberg equilibrium for the BDNF polymorphism (χ^2=0.6421; p=0.001). Since one copy of Met allele might induce functional change, further analysis was carried out in recessive model. Data in Table 2 reveal that the social-demographic characteristics evaluated did not differ according to the genotype distribution. However, the Met allele was significantly associated with higher risk to develop of GAD (p=0.029).

Although no differences in serum BDNF levels were found according to the GAD diagnostic or genotypes, the Met66 allele was significantly associated with increase in serum BDNF levels (8.2±2.35 ng/mL), when compared to the Val/Val genotype (6.9±2.62 ng/mL) in the GAD subjects (p=0.038). In the control group the Met66 allele have no effect on serum BDNF levels (7.4±2.25 ng/mL) in comparison to Val/Val genotype (7.5±2.35 ng/mL) (p=0.842).

4. Discussion

The present study showed that Met66 allele of the rs6265 polymorphism in BDNF gene convenes risk for GAD. Although, BDNF serum levels did not differ between diagnostic groups and genotype distribution, after the stratification of the sample according to clinical diagnostic, Met66 variant was significantly associated with increased serum BDNF levels in subjects with GAD diagnosis.

Studies have suggested that peripheral BDNF levels might be a biomarker for neuropsychiatric disorder, although there is no consensus in the literature[15,30,33,35]. Until now, few clinical studies have investigated the relation between BDNF protein levels and anxiety disorders. In this context, peripheral level of BDNF were found lower in subjects with posttraumatic stress disorder (PTSD) and obsessive-compulsive disorder (OCD) when compared to control groups without any anxiety disorders[29,30,34,35]. In fact, a recent meta-analysis showed that peripheral BDNF are likely the result from lower levels in OCD, rather than other anxiety disorders[33]. In addition, levels of BDNF were higher in subjects with recent trauma in comparison to the group with remote trauma. It is important to highlight that the protein levels decrease over the time[35]. Additionally, recent studies failed to found differences in BDNF protein levels in relation to distinct phenotypical categories of anxiety disorders[36,37]. Similarly, our results showed that BDNF protein levels are not associated with GAD[37,38].

Besides the evaluation of peripheral BDNF levels, a series of studies have been investigated the potential risk of the functional Val66Met polymorphism in the BDNF gene with anxiety disorders[16,17,27,28]. Some studies have reported that Met66 variant of the polymorphism increased the risk for developing other psychiatric disorders, such as mood disorders, eating disorders, and schizophrenia[39,15]. Our results reveal, for the first time, that Met66 allele is associated with GAD and higher BDNF levels in diagnosed subjects. These results are in agreement with clinical studies in which Met66 allele convenes risk for anxiety disorders[16,28,40,41,42]. Conversely, a recent meta-analysis reported that Val66Met does not appears to be relevant for the anxiety disorders[27], and the literature still limited in regard to the association [16,42,43,44,45,46]. According to our results, is important to highlight that the Met66 variant was identified by induce the

increase in BDNF protein levels in healthy subjects[47]. Similarly, another study showed that higher levels or BDNF were associated with Met66 allele just in healthy male[48]. These findings suggest a compensatory mechanism for low efficiency of intracellular BDNF trafficking and secretion reported to Met66 allele.

The BDNF is an important factor involved in the memory processing in anxiety and mood disorders[22,28]. Based in animal studies, the increase in BDNF levels is implicated in emotional and fear memory formation and consolidation through increased BDNF gene expression and activation of its high-affinity TrkB receptor in the amygdala, hippocampus and prefrontal cortex[49]. Evidence shows that BDNF-induced changes in the hippocampus may play a role in stress-related pathology, demonstrating that BDNF infusion leads to anxiogenic effect in rats by enhancing the serotonergic signaling mediated by 5-HT1A receptors[50]. Therefore, although the Met66 variant has been hypothesized to decrease the BDNF activity dependent secretion but not the constitutive one, the upregulation of peripheral BDNF concentrations in the carriers of the Met66 allele might compensate a defective intracellular protein signaling[17].

The studies correlating BDNF and anxiety disorders are yet very controversial, since there is heterogeneity in sample characteristics, study methodology, measures of outcome, different units of measurement, and the relatively small number of participants in most of them. Furthermore, the definition of anxiety phenotypes might also be the cause of the inconsistent results.

Conclusion

The association of Met66 allele with GAD and the levels of serum BDNF, provides evidence that this polymorphism could be a marker for the disease. Besides that, the increase in BDNF protein levels might reflect a compensatory elevation in response to a higher risk of psychopathology due to carrying the less functional BDNF allele. It is important to highlight that our study has the methodological strength by evaluating drug-naıve young subjects experiencing the early stages of these illnesses.

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Disclosures

The authors of this paper do not have any potential conflict of interests in connection with this manuscript.

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Tables:

Table 1. Socio-demographic and clinical characteristics of the sample according to the clinical diagnostic

	Controls	**Generalized anxiety disorder**	**p *value***
Female Gender	334 (53.9%)	77 (74.8%)	0.001
Caucasian Ethnicity	485 (78.2%)	75 (72.8%)	0.224
Age (years)	26.13±5.33	26.97±5.11	0.135
Brazilian Economic index			0.003
1 (minor)	198 (32.0%)	47 (45.6%)	
2 (midle)	206 (33.3%)	33 (32.0%)	
3 (highest)	216 (34.8%)	24 (22.3%)	
Body Mass Index (BMI) (Kg/m²)	25.89±4.60	25.08±4.21	0.095
Alcohol use	53 (8.5%)	13 (12.6%)	0.184
Tobacco use	121 (19.7%)	42 (42.0%)	0.001
Psychiatric medication	30 (4.8%)	17 (16.5%)	0.001
Psychiatric Variables			
Major Depressive	59 (9.5%)	39 (37.9%)	0.001
Bipolar Disorder	53 (10.2%)	35 (42.7%)	0.001
Social Phobia	14 (2.3%)	24 (23.3%)	0.001
Agoraphobia	66 (10.6%)	53 (51.5%)	0.001
Obsessive Compulsive Disorder	10 (1.6%)	23 (22.3%)	0.001
Post traumatic Stress	3 (0.5%)	16 (15.5%)	0.001
Panic Disorder	6 (1.0%)	25 (24.3%)	0.001
Serum BDNF levels (ng/mL)	7.40±2.28	7.37±2.59	0.652
Total	**620**	**103**	**723**

Displayed as mean ± standard deviation or n and %. p values ≤ 0.05 were considered significant for differences in socio demographic characteristics between clinical diagnoses of current depression. The differences were evaluated by Student t *test*, and X^2, as appropriated.

Table 2. Demographic and clinical characteristics distributed according to BDNF genotype

		GG	GA/AA	p *value*
Female Gender		295 (56.7%)	116 (57.1%)	0.920
Caucasian Ethnicity		400 (76.9%)	160 (78.8%)	0.584
Age (years)		26.22±5.27	26.32±5.40	0.816
BMI (Kg/m²)		25.85 ±4.59	25.57±4.48	0.435
Serum BDNF levels (ng/mL)		7.30±2.30	7.65±2.38	0.222
Psychiatric Variables				
Major Depressive	Yes	71 (72.4%)	27 (27.6%)	0.901
	No	449 (71.8%)	176 (28.2%)	
Bipolar Disorder	Yes	63 (71.6%)	25 (28.4%)	0.971
	No	367 (71.4%)	147 (28.6%)	
Agoraphobia Atual	Yes	84 (70.6%)	35 (29.4%)	0,723
	No	436 (72.2%)	168 (27.8%)	
Social Phobia	Yes	24 (63.2%)	14 (36.8%)	0.217
	No	496 (72.4%)	189 (27.6%)	
OCD	Yes	22 (66.7%)	11 (33.3%)	0.492
	No	498 (72.2%)	192 (27.8%)	
PTSD	Yes	12 (63.2%)	7 (36.8%)	0.389
	No	508 (72.2%)	196 (27.8%)	
Panic Disorder	Yes	18 (58.1%)	13 (41.9%)	0.144
	No	494 (72.8%)	185 (27.2%)	
GAD	Yes	65 (63.1%)	38 (36.9%)	0.029
	No	456 (73.5%)	164 (26.5%)	
Total		**521**	**202**	**723**

Displayed as mean ± standard deviation or n and %. p values ≤ 0.05 were considered significant for differences in socio demographic characteristics between clinical diagnoses of current depression. The differences were evaluated by Student t *test*, and X^2, as appropriated. OCD: Obsessive Compulsive Disorder; PTSD: Pos Traumatic Stress Disorder

Figure 1

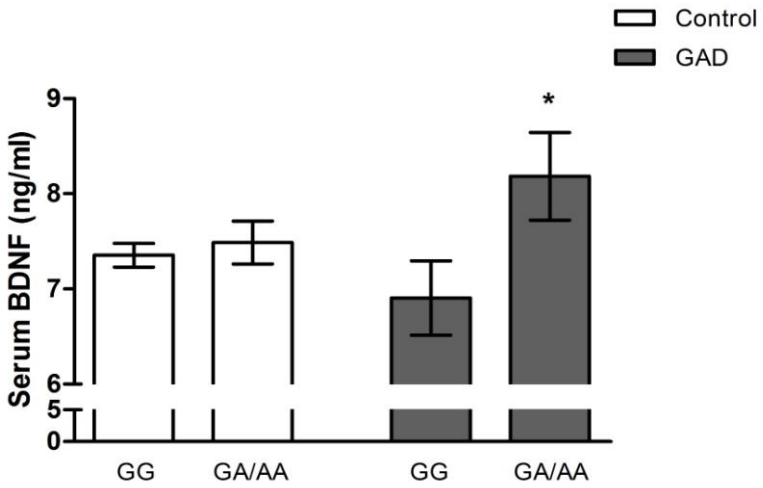

Figure Legend:

Figure 1. Serum BDNF levels according genotype distribution after stratification of sample by GAD diagnosis. White bars represents control group and grey bars represents GAD subjects. Data are presented as mean ± S.E.M evaluated by One Way Anova followed by Tukey post-hoc. * $p \leq 0.05$. GAD: Generalized Anxiety Disorder