European Archives of Psychiatry and Clinical Neuroscience (2021) 271:1141–1148
https://doi.org/10.1007/s00406-019-01051-8

ORIGINAL PAPER

Leptin polymorphism rs3828942: risk for anxiety disorders?

Pamela Silva Vitória Salerno[1] · Clarissa Ribeiro Bastos[1] · Ariadni Peres[1] · Ana Paula Ardais[1] · Marta Gazal[1] · Karen Jansen[1] · Luciano Dias de Mattos Souza[1] · Ricardo Azevedo da Silva[1] · Manuella Pinto Kaster[2] · Diogo Rizzato Lara[3] · Gabriele Ghisleni[1]

Received: 6 March 2019 / Accepted: 7 August 2019 / Published online: 16 August 2019

Abstract

Leptin is an anorexigenic hormone well recognized by its role in mediating energy homeostasis. Recently, leptin has been associated with psychiatric disorders and interestingly, leptin treatment has shown antidepressant and anxiolytic effects. We examined the association of leptin levels and leptin (*LEP*) gene rs3828942 polymorphism with anxiety disorders considering sex differences. A cross-sectional population-based study, including 1067 young adults, of whom 291 presented anxiety disorders diagnosed by the Mini International Neuropsychiatric Interview (MINI 5.0). The rs3828942 polymorphism was genotyped by real-time PCR and ELISA measured leptin levels. Leptin levels were not associated with anxiety disorders after adjusting for sex and body mass index (BMI) [$\beta = -0.009$ (-0.090–0.072); $p = 0.832$]. The distribution of rs3828942 genotypes was not associated with anxiety disorders. However, in a sex-stratified sample, the A-allele of rs3828942 polymorphism was associated with risk for GAD in women even when adjusting for confounding variables [OR = 1.87 (1.17–2.98); $p = 0.008$]. In a subsample of 202 individuals with GAD and control matched by sex and BMI, results suggest an interaction between genotypes and GAD diagnosis based on leptin levels only in the male group [F (1, 54) = 6.464; $p = 0.0139$]. Leptin is suggested to be related with the neurobiology of anxiety disorders in a sex-dependent manner since women carrying the A-allele of *LEP* rs3828942 present a higher risk for GAD, while leptin levels seem to be lower in men with GAD carrying A-allele. Studies on the relationship between leptin polymorphisms and levels are scarce and, therefore, further research is necessary.

Keywords Leptin · Leptin polymorphism · Anxiety disorders · Generalized anxiety disorder

Introduction

Anxiety disorders are chronic and functionally disabling conditions that are among the most frequent psychiatric disorders. Recent data from the World Health Organization (WHO) have pointed out that the segment of the global population with a higher prevalence of anxiety disorders is women [1]. Although particular symptoms and signs lead to differential categories of anxiety disorders, such as the duration and severity of each clinical diagnosis (e.g., generalized anxiety disorder (GAD), agoraphobia, social phobia), some symptoms, such as chronic and persistent worry, tension, sleep disturbances, and cognitive impairment are similar [1, 2].

Women have greater susceptibility to develop depression and anxiety disorders since they are more sensitive to psychological stress [3–5]. Genetic factors and hormonal regulation may differentially influence women's environmental

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00406-019-01051-8) contains supplementary material, which is available to authorized users.

✉ Gabriele Ghisleni
ghisleni.g@gmail.com

[1] Institute of Health Sciences, Department of Health and Behavior, Catholic University of Pelotas - UCPel, Rua Gonçalves Chaves 373, Sala 324, Pelotas, RS 96010-280, Brazil

[2] Department of Biochemistry, Federal University of Santa Catarina, Florianopolis, SC, Brazil

[3] Department of Cellular and Molecular Biology, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil

responses to stress [6–8]. In this regard, leptin is a polypeptide hormone exponentially synthesized in proportion to the amount of adipose tissue, which presents a higher secretion in women than in men [9]. Leptin has a well-established role in regulating food intake and body weight [10–13] and recently, an additional role in mood and emotion regulation has been demonstrated [14–17].

Indeed, in animal models of depression, chronic stress has been associated with decreased leptin levels and leptin administration was able to reduce depressive and anxiety-like behaviors [14, 17–19], and has facilitated the extinction of conditioned fear responses [17–20]. In addition, leptin-knockout mice have exhibited marked increases in depression and anxiety-related behaviors, which can be reverted by leptin administration [21–24]. In the clinical field, leptin deregulation has been associated with depressive disorders although the findings have been conflicting, showing both higher and lower levels of leptin, or none association with the disorder [25–27]. Within the context of anxiety disorders, results found in the literature are scarce. Furthermore, studies investigating the association of genetic variants in *LEP* and *LEPR* gene in relation to psychiatric disorders are limited, indicating the importance of further research. *LEP* gene located in chromosome 7 was first suggested to be associated with behavior disorders in the 1990s [28] and *LEP* D7S1875 repeat polymorphism was then related to depression and associated comorbidities [29]. Nowadays, *LEP* rs7799039 is one of the most well-studied polymorphisms related to weight gain in psychiatric patients under medication although data are still controversial [30–32]. Furthermore, this polymorphism has shown to be in higher linkage disequilibrium with rs3828942, a polymorphism located in intron 2, also associated with higher weight gain in schizophrenic patients [30]. However, these studies have not evaluated direct associations with psychiatric diagnoses and behavioral patterns.

Therefore, according to the emerging relevance of leptin for mood and emotion regulation, as the well-known sex differences regarding leptin secretion and anxiety prevalence, the present study aimed to investigate the association of leptin levels and single nucleotide polymorphism (SNP) rs3828942 in *LEP* gene with anxiety disorders considering sex differences in a population-based cross-sectional study.

Materials and methods

Subjects and procedures

This research was a cross-sectional population-based study, which included 1067 young adult aged 18–35 years from an urban area of Pelotas, Southern Brazil, of whom 298 had anxiety disorders. Major details of the study design were previously described [33]. Home visits were conducted in the morning when participants answered a standard questionnaire to collect sociodemographic information, lifestyle, and a blood sample was collected. Additionally, on the same day of questionnaire application, researchers measured the weight and height of participants which were used to calculate the body mass index (BMI), and individuals were categorized as presenting normal weight (BMI ≥ 18.5 and ≤ 24.9), overweight (BMI ≥ 25.0 and ≤ 29.9), and obesity (BMI ≥ 30.0) according to the Centers for Disease Control and Prevention (http://www.cdc.gov). Ethnicity was self-declared and classified as Caucasian and non-Caucasian, the last including African-American, Indian-American, and other ethnicities. To determine the presence of current and lifetime anxiety disorders including GAD, agoraphobia and social phobia, trained psychologists used a structured diagnostic instrument, the Mini International Neuropsychiatric Interview according to DSM-IV criteria (MINI. 5.0, Brazilian version/DSM IV, Medical Outcome Systems Inc., Jacksonville, FL, USA). The study was approved by the Ethics Committee of Catholic University of Pelotas, Pelotas, Brazil (Protocol number 2010/15), and all participants provided their informed consent. The inclusion criteria of the study comprised the following aspects: age between 18 and 35 years, residence place in the urban area of Pelotas, and acceptance to participate in the study signing the informed consent form. The exclusion criteria included individuals unable to respond to the interview due to physical or cognitive problems.

After obtaining the preliminary results from the association study, we also analyzed a representative subsample of 202 individuals (101 controls and 101 individuals with GAD) that were randomly selected from the population-based study and paired by sex and BMI for the evaluation of leptin serum levels according to genotypes.

Sample collection

After the clinical interview, 10 ml of blood was collected from each subject by venipuncture into a vacuum tube during morning hours (between 8:00 and 11:00 a.m.) which were used for the molecular and biochemical analysis. Blood samples from anticoagulant-free tubes were immediately centrifuged at 3500*g* for 15 min for serum isolation, which were kept frozen at − 80 °C until biochemical analysis was performed. Peripheral blood leukocytes from samples collected in EDTA tubes were used for total DNA extraction using a standardized salting-out procedure [34].

Molecular analyses

The selection of the *LEP* rs3828942 (G/A) SNP was based on (1) relevance of the *LEP* gene for cognition and stress

response; (2) previous results relating this SNP with cognitive performance; (3) allelic frequencies of the variant; (4) and technical viability. *LEP* rs3828942 SNP was genotyped using primers (TTAGAGGCTTGGCAGTCACCT GGGT**[G/A]**CAGGATACAAGGGCCTGAGCCAAAG) and probes contained in the 40 × Human Custom TaqMan Genotyping Assay (Life Technologies, Foster City, CA, USA). The reactions were conducted in a 96-well plate, in a total 5-ml reaction volume using 2 ng of genomic DNA, TaqMan Genotyping Master Mix 1 × (Applied Biosystems), and CustomTaqMan Genotyping Assay 1 ×. Then, plates were positioned in a real-time PCR thermal cycler (7500 Fast Real PCR System; Applied Biosystems) and heated for 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Fluorescence data files from each plate were analyzed using automated allele-calling software (SDS 2.0.1; Applied Biosystems).

Serum leptin levels measurement

Serum levels of leptin were measured using a specific immunoassay kit (DuoSet ELISA Development, R&D Systems, Inc., USA). The intra-assay coefficient of variation (CV) was less than 5% and the inter-assay CV was less than 10%. All samples and standards were measured in duplicate. Serum leptin level was expressed in ng/mL.

Statistical analysis

Questionnaires were typed and coded directly into the Epi-Info6 program at the interview time and statistical analysis was performed using SPSS statistical package for Windows, version 23.0. Allelic frequencies were determined by gene counting and departures from the Hardy–Weinberg equilibrium were verified using χ^2-test. Sociodemographic and clinical characteristics were compared by unpaired *t* test and χ^2-test, as appropriate. Serum leptin levels showed a skewed distribution and were logarithmically transformed before unpaired *t* test and two-way analysis of variance followed by Tukey post hoc test. Linear regression was performed to verify the association of leptin levels with anxiety disorder adjusting for sex, BMI, and age expressed as β value and 95% confidence interval. A magnitude measure of effect was calculated by binary logistic regression crudely adjusted for ethnicity or adjusted for BMI, age, ethnicity, and sex when appropriate, and presented as odds ratio (OR) and 95% confidence interval.

Regarding the association analysis, alpha level was corrected for multiple tests using the Bonferroni approach. Thus, significant results were those with *p* values lower than 0.008 (6 tests). All tests were two-tailed, and those tests comprising *p* values between 0.008 and 0.05 were considered as suggestive associations.

Results

Our study comprised 1067 participants, of whom 291 (27.3%) presented anxiety disorders, including GAD, social phobia, and agoraphobia. Data described in Table 1 show that women presented higher prevalence of anxiety disorders (31.7%; $p < 0.001$), and low-income individuals were affected in a higher proportion (37.2%; $p < 0.001$) as well as smokers (47.1%; $p < 0.001$). No significant differences were found according to age, ethnicity, BMI, and rs3828942 genotype distribution. Leptin levels were higher in subjects with anxiety disorders when compared to the control group (25.8 ± 33.5 and 20.18 ± 27.8, respectively; $p = 0.027$), but this association did not remain after

Table 1 Sociodemographic, clinical and biological characteristics of the sample according to anxiety disorder

Variables	Anxiety disorder		*p* value
	No	Yes	
Gender			< 0.001
Female	424 (68.3%)	197 (31.7%)	
Male	351 (78.9%)	94 (21.1%)	
Age	26.00 ± 5.3	26.0 ± 5.2	0.865
BMI	26.05 ± 5.3	26.11 ± 5.8	0.695
Ethnicity			0.139
Caucasian	595 (73.9%)	210 (26.1%)	
No caucasian	180 (69.0%)	81 (31.0%)	
Socioeconomic class			< 0.001
Low	135 (62.8%)	80 (37.2%)	
Intermediate	386 (72.1%)	149 (27.9%)	
High	254 (80.6%)	61 (19.4%)	
Smoke			< 0.001
Yes	129 (52.9%)	115 (47.1%)	
No	645 (78.9%)	172 (21.1%)	
Leptin levels (ng/ml)	20.18 ± 27.8	25.8 ± 33.5	0.027
rs3828942 genotypes			0.689
GG	263 (33.9%)	98 (33.7%)	
AG	405 (52.2%)	158 (54.3%)	
AA	108 (13.9%)	35 (12.0%)	
Genotypes (dominant model)			1.000
GG	263 (33.9%)	98 (33.7%)	
AG/AA	513 (66.1%)	193 (66.3%)	
Genotypes (recessive model)			0.480
GG/AG	668 (86.1%)	256 (88.0%)	
AA	108 (13.9%)	35 (12.0%)	
Total	776	291	

Values are expressed as mean ± SD or *n* (%). Values of $p < 0.05$ were considered statistically significant. Anxiety disorder includes generalized anxiety disorder, agoraphobia and social phobia. Data were analyzed by Student *t* test or χ^2-square test

the linear regression adjusted for sex and BMI [$\beta = -0.009$ (-0.090–0.072); $p = 0.832$].

The genotype distribution of rs3828942 SNP was in agreement with the distribution predicted by the Hardy–Weinberg equilibrium ($\chi^2 = 0.798$; $p = 0.371$). Distribution of genotypes differed according to ethnicity with a higher proportion of AA genotype (15.3%) in Caucasian individuals compared to non-Caucasian (7.7%; $p = 0.001$). However, other sociodemographic and biological characteristics such as sex, socioeconomic level, BMI, and leptin levels did not differ according to genotypes distribution (data not shown).

Our results revealed no association between the genotypes distribution of *LEP* rs3828942 polymorphism according to the diagnosis of GAD ($p = 0.269$), agoraphobia ($p = 0.171$), and social phobia ($p = 0.174$) (data not shown). Data described in Table 2 showed no association between rs3828942 polymorphism and agoraphobia or social phobia using both dominant and recessive models, while a suggestive association was demonstrated between the dominant model and GAD with A-allele conferring risk [OR = 1.47 (1.008–2.156); $p = 0.045$]. In addition, subjects with GAD presented higher levels of leptin compared to controls (27.66 ± 35.0 and 20.59 ± 27.8, respectively; $p = 0.030$), while no differences were observed for leptin levels considering social phobia or agoraphobia diagnosis (data not shown). However, after adjusting for sex and BMI in the linear regression, leptin levels and GAD did not remain associated [$\beta = 0.002$ (-0.099–0.103); $p = 0.964$].

Moreover, considering the higher prevalence of anxiety disorders in women, we analyzed the dominant model according to anxiety diagnosis after sex stratification (Table 3). Our results showed that A-allele of rs3828942 polymorphism confers risk for GAD in women compared to controls even after adjusting the logistic regression for age, ethnicity, and BMI [OR = 1.87 (1.17–2.98); $p = 0.008$), and no association was observed for men ($p = 0.759$) as well (Table 3). No significant influences of rs3828942 genotypes

Table 2 Association analysis between the leptin rs3828942 SNP and anxiety disorders

	GAD		Agoraphobia		Phobia social	
	OR (95% CI)/*p**	OR (95% CI)/*p***	OR (95% CI)/*p**	OR (95% CI)/*p***	OR (95% CI)/*p**	OR (95% CI)/*p***
rs3828942 Genotypes dominant model						
GG	1	1	1	1	1	1
GA/AA	1.33 (0.931–1.925)/0.116	1.47 (1.008–2.156)/**0.045**	0.85 (0.616–1.185)/0.346	0.87 (0.625–1.222)/0.431	1.38 (0.783–2.432)/0.265	1.33 (0.747–2.368)/0.332
Recessive model						
GG/AG	1	1	1	1	1	1
AA	0.99 (0.611–1.629)/0.993	1.10 (0.661–1.830)/0.714	0.65 (0.388–1.104)/0.112	0.61 (0.355–1.073)/0.087	0.55 (0.195–1.572)/0.267	0.55 (0.195–1.572)/0.267

The significant and suggestive results are denoted in bold

*Binary logistic regression adjusted by ethnicity

**Binary logistic regression adjusted by ethnicity, age, sex and BMI and represented as OR (95% CI)/*p*

Table 3 Association analysis between the leptin rs3828942 SNP and anxiety disorders in sex-stratified sample

	GAD		Agoraphobia		Phobia social	
	OR (95% CI)/*p**	OR (95% CI)/*p***	OR (95% CI)/*p**	OR (95% CI)/*p***	OR (95% CI)/*p**	OR (95% CI)/*p***
Woman rs3828942 dominant model						
GG	1	1	1	1	1	1
GA/AA	1.76 (1.13–2.75)/**0.013**	1.87 (1.17–2.98)/**0.008**	1.08 (0.71–1.63)/0.717	1.10(0.72–1.68)/0.659	1.80 (0.93–3.49)/0.082	1.63 (0.84–3.20)/0.149
Men rs3828942 dominant model						
GG	1	1	1	1	1	1
GA/AA	0.807 (0.42–1.54)/0.516	0.90 (0.46–1.75)/0.759	0.59 (0.35–1.01)/0.056	0.63 (0.36–1.09)/0.100	0.68 (0.21–2.17)/0.511	0. 68 (0.21–2.21)/0.530

The significant and suggestive results are denoted in bold

*Binary logistic regression adjusted by ethnicity

**Binary logistic regression adjusted by ethnicity, age, sex and BMI and represented as OR (95% CI)/*p*

were observed for agoraphobia and social phobia for both women and men (Table 3). In the stratified sample by sex, leptin levels did not differ according to any anxiety diagnosis (data not shown).

In view of the results showing an association between rs3828942 SNP and GAD in women, we performed in a subsample of individuals, an analysis of the interaction between *LEP* rs3828942 genotypes (GG and GA/AA) and GAD considering leptin levels, which revealed no significant interaction [$F(1, 205) = 1.569$; $p = 0.2117$] (Fig. 1a). However, when the analysis was performed in a sex-stratified sample, a suggestive interaction was observed in the male group, where subjects with GAD carrying the GG genotype presented lower leptin levels than GG genotype control subjects [$F(1, 54) = 6.464$; $p = 0.0139$] with no differences for women [$F(1, 147) = 0.2158$; $p = 0.642$] (Fig. 1b).

Discussion

Beyond the leptin well-recognized function in metabolism homeostasis, our study sought to evaluate the additional role of leptin in anxiety disorders, since recent evidence of pre-clinical and clinical studies indicate to its regulation in depressive and anxiety behavior. Here, we demonstrated that leptin levels in anxiety disorders and, specifically GAD, are dependent on sex and BMI. Moreover, *LEP* rs3828942 A-allele seems to confer a risk factor for GAD in a sex-dependent manner.

Genetic mouse models have highlighted *LEP* and *LEPR* genes as important tools to understand the molecular and pathophysiological processes involved in complex human diseases such as psychiatric disorders. Interestingly, leptin-deficient (ob/ob) mice presented anxious and depressive-like behavior [21–23], which were reversed by administrating leptin [22, 23]. Leptin also showed a potential antidepressant-like activity as measured by immobility time in forced swimming test in mice which revealed an increasing *c*-fos mRNA expression in the hippocampus [35]. In addition, rats exposed to chronic unpredictable stress (CUS) exhibited a hedonic-like deficit in sucrose preference test and low plasma leptin levels [35]. Furthermore, leptin administration in rats chronically stressed was also effective in reversing anxious-like profiles and increasing serum corticosterone levels in a dose-dependent manner [18]. Within the context of fear conditioning, an animal model for anxiety disorders, both systemic and intra-lateral amygdala administration of leptin facilitates the extinction of conditioned fear responses reducing anxiety symptoms [20]. In fact, leptin and its receptors are widely distributed in brain areas which are involved in the control of mood and emotion, such as the limbic structures. The signaling operated by leptin has

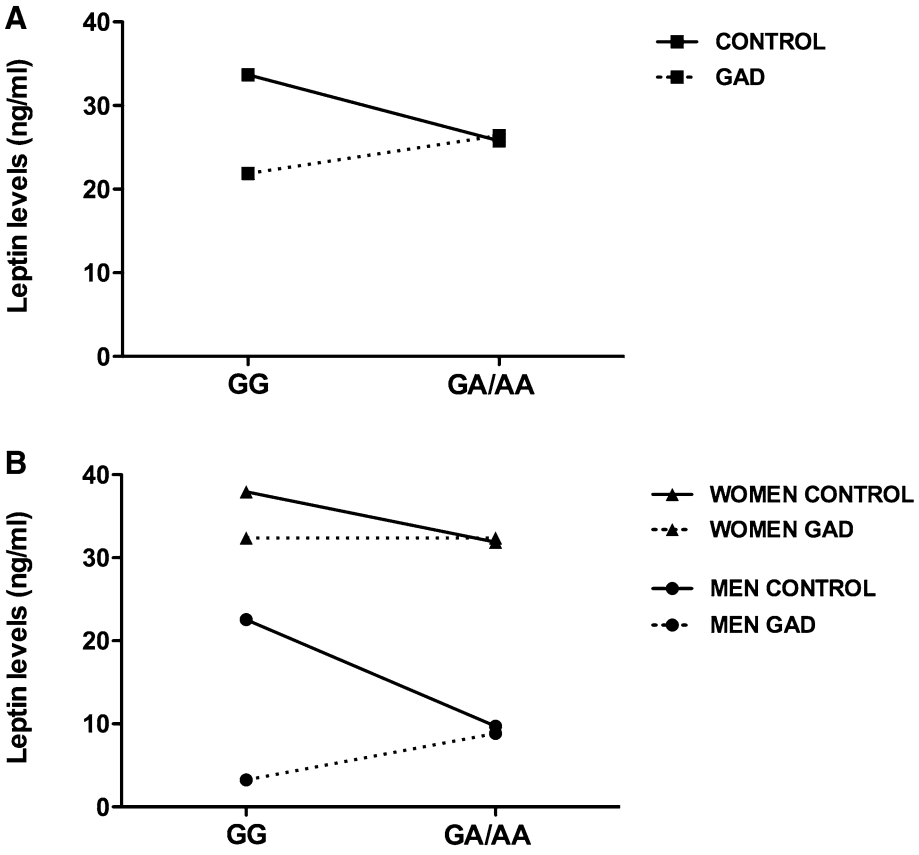

Fig. 1 **a** Serum leptin levels according to rs3828942 genotypes distribution in GAD (GG = 25; GA/AA = 79) and control subjects (GG = 36; GA/AA = 69). **b** Serum leptin levels of women and men expressed according to rs3828942 genotypes distribution in GAD (women-GG = 16; women-GA/AA = 59; men-GG = 9; men-GA/AA = 20) and control subjects (women-GG = 26; women-GA/AA = 50; men-GG = 10; men-GA/AA = 19). Leptin levels were expressed in ng/ml as mean values. Values of $p \leq 0.05$ were considered statistically significant as evaluated by two-way ANOVA followed by Tukey post hoc test. GAD = Generalized Anxiety Disorder

important functions in the modulation of cognitive processes, neurogenesis, synaptogenesis, and neuroprotection [15]. Therefore, preclinical results describing the anxiolytic and antidepressant effects of leptin have aroused the interest of the scientific community in carrying out further clinical research.

From a clinical perspective, data evaluating leptin signaling in anxiety disorders are still limited. Epidemiological studies have reported controversial results regarding depressive disorders, showing high [36, 37] and low [33, 38, 39] peripheral leptin levels or no significant differences between depressed and non-depressed subjects [40]. Although data concerning anxiety are scarce in the literature, a clinical study evaluating reproductive-aged women with mild depression and anxiety showed lower levels of leptin when compared to healthy volunteers [41]. Conversely, our results demonstrated no consistent data regarding the association between leptin levels and anxiety disorders using sex and BMI adjusted analysis. As both parameters directly influence leptin secretion, it is important to conduct further studies according to differential diagnosis. While agoraphobia is characterized by intense fear or anxiety triggered by actual or predicted exposure to various situations, social phobia differs from it concerning the type of situations that elicit fear and anxiety. On the other hand, GAD symptoms are expressed by anxiety and excessive worry about various events or activities. Moreover, the risk factors and prognosis for these disorders differ according to individual temperament, environment, genetics, and physiological factors (DSM-V).

Studies with genetic variants have shown that polymorphisms in the *LEP* or *LEPR* genes have been extensively investigated regarding metabolic disorders and weight gain [42, 43]. The G-allele of rs3828942 SNP in the *LEP* gene was associated with higher fat mass index in adolescents who had a lower ponderal index at birth [44]. Conversely, our findings demonstrated no association of rs3828942 variants with BMI in the general sample. Currently, there are few studies investigating the potential association of SNPs in leptin regulation in the context of psychiatric disorders. In this regard, SNPs already studied in this pathway have been considered as potential pharmacogenetic markers for weight gain associated with antipsychotics, such as the SNPs rs1137101, rs8179183, rs1805134, and rs1137100 [13]. The rs7799039 SNP is located in the promoter region of *LEP* gene (in the literature usually referred to as 2548G/A) and has been found in high unbalance of binding with the rs3828942 investigated in this study, since they are in high linkage disequilibrium. However, only two studies have shown the effect of the rs3828942 variant in psychiatric patients. One of them investigating several SNPs in the *LEP* gene indicated that only rs3828942 G-allele and rs7799039 G-allele were associated with higher weight gain in schizophrenic and schizoaffective patients treated with antipsychotics [30]. Besides that, rs3828942 A-allele remained significantly associated with resistance to antidepressant treatment response, lower cognitive performance, and BMI after correction for multiple testing regarding the number of SNPs evaluated in depressive patients [45]. In our study, the genotype distribution of rs3828942 did not differ between groups for all anxiety disorders evaluated. However, after sample stratification by sex, our study revealed an association between GAD and the A-allele of *LEP* rs3828942 in women even after adjusting for confounding variables, suggesting the potential for further research on *LEP* gene SNPs and psychiatric disorders. Most importantly, our results are in agreement with previously described results for depressive patients, suggesting a risk factor associated with A-allele of rs3828942.

Although this study found no association between leptin levels and anxiety disorders, our data suggest an interaction between leptin levels and rs3828942 SNP in men, corroborating the differential effect according to sex. In this regard, we point out that sex differences in anxiety neurobiology have already been mentioned in the literature, and although women are twice as likely to develop anxiety disorders, the reasons remain unclear. Nevertheless, it is important to highlight the need for further studies to evaluate this association in larger samples. On the other hand, the inclusion of sex as a biological variable in research is absolutely essential for improving our understanding of disease mechanisms contributing to risk and resilience for anxiety disorders [46].

The present results show that the association of leptin levels with anxiety disorders is dependent on sex and BMI and that female individual carrying the rs3828942 A-allele of *LEP* gene present a higher risk of developing GAD. Most importantly, the results described here indicate the involvement of the leptin system in the neurobiology of anxiety in a sex-dependent manner. The importance of our study in assessing leptin levels and leptin genetic variants in a population-based study, along with the limiting and inconsistent findings in this research field, highlights that further studies are required to elucidate our findings.

Despite the role of leptin signaling in anxiety disorders which has been suggested in this study, our findings should be considered in light of some limiting factors. First, the study had a cross-sectional design and, therefore, a longitudinal evaluation would be more appropriate to better understand the impact of this polymorphism on leptin levels and susceptibility to anxiety. Second, we did not research the severity of anxiety symptoms. Third, food intake was not controlled in blood sample collection, and fourth, the small sample size in each group might have limited in particular the analysis of leptin levels. Regardless of these limitations, the methodological strengths of this study should be

considered when using a higher population-based sample of young subjects for leptin evaluation.

Acknowledgements We would like to thank Editage (http://www.editage.com) for English language editing.

Author contributions All authors mentioned in the paper have significantly contributed to the research. RAS, KJ, LDS, and DRL conceived and supervised the clinical evaluation. MPK and GG supervised the collection and processing of biological samples. PVS, CRB, and AP performed the DNA extraction and genotyping and MG and PVS performed leptin level measurements. PVS, CRB, APA, AP, GG, and MPK performed statistical analysis and wrote the manuscript.

Funding This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) and PRONEX-FAPERGS (08/2009 - Pronex 10/0055-0). RS, KJ, LDS, MPK, and DRL are CNPq Research Fellows. PVS, CRB, and APA received a fellowship from CAPES.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Statement of ethics The study protocol was approved by the Ethics Committee of Catholic University of Pelotas, Pelotas, Brazil (Protocol number 2010/15). All participants signed the written informed consent.

References

1. World Health Organization (2017) Depression and other common mental disorders: global health estimates. License: CC BY-NC-SA 3.0 IGO. http://www.who.int/iris/handle/10665/254610. Accessed 10 Jan 2019
2. American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders (DSM-5®). American Psychiatric Pub
3. Bale TL, Baram TZ, Brown AS, Goldstein JM, Insel TR, McCarthy M et al (2010) Early life programming and neurodevelopmental disorders. Biol Psychiat 68:314–319. https://doi.org/10.1016/j.biopsych.2010.05.028
4. Kessler RC, Ormel J, Demler O, Stang PE (2003) Comorbid mental disorders account for the role impairment of commonly occurring chronic physical disorders: results from the National Comorbidity Survey. J Occup Environ Med 45:1257–1266. https://doi.org/10.1097/01.jom.0000100000.70011.bb
5. Pryce CR, Fuchs E (2017) Chronic psychosocial stressors in adulthood: studies in mice, rats and tree shrews. Neurobiol Stress 6:94–103. https://doi.org/10.1016/j.ynstr.2016.10.001
6. Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S et al (1994) Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States: results from the National Comorbidity Survey. Arch Gen Psychiatry 51:8–19. https://doi.org/10.1001/archpsyc.1994.03950010008002
7. Craske MG, Stein MB, Eley TC, Milad, Holmes A, Rapee RM et al (2017) Anxiety disorders. Nat Rev Dis Primers 3:17024. https://doi.org/10.1038/nrdp.2017.24
8. Remes O, Wainwright N, Surtees P, Lafortune L, Khaw KT, Brayne C (2017) Sex differences in the association between area deprivation and generalised anxiety disorder: British population study. BMJ Open 7:e013590. https://doi.org/10.1136/bmjopen-2016-013590
9. Wiesner G, Vaz M, Collier G, Seals D, Kaye D, Jennings G et al (1999) Leptin is released from the human brain: influence of adiposity and gender. J Clin Endocrinol Metab 84:2270–2274. https://doi.org/10.1210/jcem.84.7.5854
10. Ostlund RE Jr, Yang JW, Klein S, Gingerich R (1996) Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. J Clin Endocrinol Metab 81:3909–3913. https://doi.org/10.1210/jcem.81.11.8923837
11. Van Aggel-Leijssen DPC, Van Baak MA, Tenenbaum R, Campfield LA, Saris WHM (1999) Regulation of average 24 h human plasma leptin level; the influence of exercise and physiological changes in energy balance. Int J Obes 23:151. https://doi.org/10.1038/sj.ijo.0800784
12. Ahima RS, Osei SY (2004) Leptin signaling. Physiol Behav 81:223–241. https://doi.org/10.1016/j.physbeh.2004.02.014
13. Lee AK, Bishop JR (2011) Pharmacogenetics of leptin in antipsychotic-associated weight gain and obesity-related complications. Pharmacogenomics 12:999–1016. https://doi.org/10.2217/pgs.11.45
14. Liu J, Garza JC, Bronner J, Kim CS, Zhang W, Lu XY (2010) Acute administration of leptin produces anxiolytic-like effects: a comparison with fluoxetine. Psychopharmacology 207:535–545. https://doi.org/10.1007/s00213-009-1684-3
15. Dodd S, Maes M, Anderson G, Dean OM, Moylan S, Berk M (2013) Putative neuroprotective agents in neuropsychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry 42:135–145. https://doi.org/10.1016/j.pnpbp.2012.11.007
16. Haleem DJ (2014) Investigations into the involvement of leptin in responses to stress. Behav Pharmacol 25:384–397. https://doi.org/10.1097/FBP.0000000000000050
17. Haleem DJ, Haider S, Perveen T, Haleem MA (2015) Serum leptin and cortisol, related to acutely perceived academic examination stress and performance in female university students. Appl Psychophysiol Biofeedback 40:305–312. https://doi.org/10.1007/s10484-015-9301-1
18. Haque Z, Akbar N, Yasmin F, Haleem MA, Haleem DJ (2013) Inhibition of immobilization stress-induced anorexia, behavioral deficits, and plasma corticosterone secretion by injected leptin in rats. Stress 16:353–362. https://doi.org/10.3109/10253890.2012.736047
19. Alò R, Avolio E, Mele M, Fazzari G, Carelli A, Facciolo RM, Canonaco M (2017) Role of leptin and orexin-a within the suprachiasmatic nucleus on anxiety-like behaviors in hamsters. Mol Neurobiol 54:2674–2684. https://doi.org/10.1007/s12035-016-98479
20. Wang W, Liu SL, Li K, Chen Y, Jiang B, Li YK et al (2015) Leptin: a potential anxiolytic by facilitation of fear extinction. CNS Neurosci Ther 21:425–434. https://doi.org/10.1111/cns.12375
21. Asakawa A, Inui A, Inui T, Katsuura G, Fujino MA, Kasuga M (2003) Leptin treatment ameliorates anxiety in ob/ob obese mice. J Diabetes Complicat 17:105–107. https://doi.org/10.1016/S1056-8727(02)00185-X
22. Finger BC, Dinan TG, Cryan JF (2010) Leptin-deficient mice retain normal appetitive spatial learning yet exhibit marked increases in anxiety-related behaviours. Psychopharmacology 210:559–568. https://doi.org/10.1007/s00213-010-1858-z
23. Yamada N, Katsuura G, Ochi Y, Ebihara K, Kusakabe T, Hosoda K, Nakao K (2011) Impaired CNS leptin action is implicated in depression associated with obesity. Endocrinology 152:2634–2643. https://doi.org/10.1210/en.2011-0004
24. Guo M, Lu Y, Garza JC, Li Y, Chua SC, Zhang W et al (2012) Forebrain glutamatergic neurons mediate leptin action on

depression-like behaviors and synaptic depression. Transl Psychiatry 2:e83. https://doi.org/10.1038/tp.2012.9
25. Kraus T, Haack M, Schuld A, Hinze-Selch D, Pollmächer T (2001) Low leptin levels but Norma body mass indices in patients with depression or schizophrenia. Neuroendocrinology 73:243–247. https://doi.org/10.1159/000054641
26. Lawson EA, Miller KK, Blum JI, Meenaghan E, Misra M, Eddy KT et al (2012) Leptin levels are associated with decreased depressive symptoms in women across the weight spectrum, independent of body fat. Clin Endocrinol 76:520–525. https://doi.org/10.1111/j.1365-2265.2011.04182.x
27. Milaneschi Y, Lamers F, Bot M, Drent ML, Penninx BW (2017) Leptin dysregulation is specifically associated with major depression with atypical features: evidence for a mechanism connecting obesity and depression. Biol Psychiatry 81:807–814. https://doi.org/10.1016/j.biopsych.2015.10.023
28. Comings DE, Rosenthal RJ, Lesieur HR, Rugle LJ, Muhleman D, Chiu C et al (1996) A study of the dopamine D2 receptor gene in pathological gambling. Pharmacogenetics 6:223–234. https://doi.org/10.1097/00008571-199606000-00004
29. Kapoor M, Kapur S, Mehra S, Dube U, Sharad S, Sidhu S (2009) Genetic variation in D7S1875 repeat polymorphism of leptin gene is associated with increased risk for depression: a case-control study from India. Depress Anxiety 26:791–795. https://doi.org/10.1002/da.20570
30. Brandl EJ, Frydrychowicz C, Tiwari AK, Lett TAP, Kitzrow W, Büttner S et al (2012) Association study of polymorphisms in leptin and leptin receptor genes with antipsychotic-induced body weight gain. Prog Neuro-Psychopharmacol Biol Psychiatry. https://doi.org/10.1016/j.pnpbp.2012.03.001
31. Kuo PH, Kao CF, Chen PY, Chen CH, Tsai YS, Lu ML, Huang MC (2011) Polymorphisms of INSIG2, MC4R, and LEP are associated with obesity-and metabolic-related traits in schizophrenic patients. J Clin Psychopharmacol 31:705–711. https://doi.org/10.1097/JCP.0b013e318234ee84
32. Gregoor JG, van der Weide J, Loovers HM, van Megen HJ, Egberts TC, Heerdink ER (2010) Association between LEP and LEPR gene polymorphisms and dyslipidemia in patients using atypical antipsychotic medication. Psychiatr Genet 20:311–316. https://doi.org/10.1097/YPG.0b013e32833b6378
33. Cordas G, Gazal M, Schuch EM, Spessato BC, Branco J, Jansen K et al (2015) Leptin in depressive episodes: is there a difference between unipolar and bipolar depression? Neuroendocrinology 101:82–86. https://doi.org/10.1159/000371803
34. Lahiri DK, Nurnberger JI Jr (1991) A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 19:5444
35. Lu XY, Kim CS, Frazer A, Zhang W (2006) Leptin: a potential novel antidepressant. Proc Natl Acad Sci 103:1593–1598. https://doi.org/10.1073/pnas.0508901103
36. Häfner S, Zierer A, Emeny RT, Thorand B, Herder C, Koenig W et al (2011) Social isolation and depressed mood are associated with elevated serum leptin levels in men but not in women. Psychoneuroendocrinology 36:200–209. https://doi.org/10.1016/j.psyneuen.2010.07.009
37. Pasco JA, Jacka FN, Williams LJ, Henry MJ, Nicholson GC, Kotowicz MA, Berk M (2008) Leptin in depressed women: cross-sectional and longitudinal data from an epidemiologic study. J Affect Disord 107:221–225. https://doi.org/10.1016/j.jad.2007.07.024
38. Jow GM, Yang TT, Chen CL (2006) Leptin and cholesterol levels are low in major depressive disorder, but high in schizophrenia. J Affect Disord 90:21–27. https://doi.org/10.1016/j.jad.2005.09.015
39. Westling S, Ahrén B, Träskman-Bendz L, Westrin Å (2004) Low CSF leptin in female suicide attempters with major depression. J Affect Disord 81:41–48. https://doi.org/10.1016/j.jad.2003.07.002
40. Cao B, Chen Y, Brietzke E, Cha D, Shaukat A, Pan Z et al (2018) Leptin and adiponectin levels in major depressive disorder: a systematic review and meta-analysis. J Affect Disord 238:101–110. https://doi.org/10.1016/j.jad.2018.05.008
41. Yoshida-Komiya H, Takano K, Fujimori K, Niwa SI (2014) Plasma levels of leptin in reproductive-aged women with mild depressive and anxious states. Psychiatry Clin Neurosci 68:574–581. https://doi.org/10.1111/pcn.12160
42. Yang JL, Liu DX, Jiang H, Pan F, Ho CS, Ho RC (2016) The effects of high-fat-diet combined with chronic unpredictable mild stress on depression-like behavior and leptin/leprb in male rats. Sci Rep 6:35239. https://doi.org/10.1038/srep35239
43. Wu L, Sun D (2017) Leptin receptor gene polymorphism and the risk of cardiovascular disease: a systemic review and meta-analysis. Int J Environ Res Public Health 14:375. https://doi.org/10.3390/ijerph14040375
44. Labayen I, Ruiz JR, Moreno LA, Ortega FB, Beghin L, DeHenauw S et al (2011) The effect of ponderal index at birth on the relationships between common LEP and LEPR polymorphisms and adiposity in adolescents. Obesity 19:2038–2045. https://doi.org/10.1038/oby.2011.74
45. Kloiber S, Ripke S, Kohli MA, Reppermund S, Salyakina D, Uher R et al (2013) Resistance to antidepressant treatment is associated with polymorphisms in the leptin gene, decreased leptin mRNA expression, and decreased leptin serum levels. Eur Neuropsychopharmacol 23:653–662. https://doi.org/10.1016/j.euroneuro.2012.08.010
46. Bale TL, Epperson CN (2017) Sex as a biological variable: who, what, when, why, and how. Neuropsychopharmacology 42:386. https://doi.org/10.1038/npp.2016.215