Hormones and Behavior 97 (2018) 14–17

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Implication of galanin gene *rs948854* polymorphism in depressive symptoms in adolescents

Fernanda da Conceição Machado[a], Livia Victorino de Souza[b], Marina Rangel[b], Zaira Palomino Jara[b], Maria do Carmo Franco[a,b,*]

[a] Division of Translational Medicine, School of Medicine, Federal University of São Paulo, São Paulo, Brazil
[b] Division of Nephrology, School of Medicine, Federal University of São Paulo, São Paulo, Brazil

ARTICLE INFO

Keywords:
Adolescents
Depression
Galanin
Single nucleotide polymorphisms

ABSTRACT

Genetic, social, and environmental conditions contribute to the development of depression, but the pathophysiological mechanisms are still unclear. Data accumulated in recent years provide significant evidence for a direct role of galanin (GAL). This study aimed to investigate the relation between SNPs in the galaninergic system and depressive symptoms in adolescents. A total of112 adolescents aged 10–18 years participated in this study. The Children Depression Inventory (CDI) was used to evaluate depressive symptoms. The effects of *rs948854* and *rs4432027* SNPs, both located within the promoter region of the GAL gene, *rs11665337* in the GALR1 receptor, and *rs8836* in the GALR2 receptor on depressive symptoms were examined. The results indicated that 30.4% of the participants had depression. We found that girls were significantly more likely to be depressive than boys. Furthermore, *rs948854* minor (G) allele was associated with depressive symptoms. Adolescents carrying the GG and AG genotype for the A/G (*rs948854*) SNP showed higher CDI scores than those carrying homozygous AA. The binomial logistic regression analysis revealed that adolescents carrying the GG genotype at SNP rs948854 had a higher likelihood of being depressive than adolescents carrying the AA or AG genotypes ($P = 0.033$). Moreover, individuals whose mothers had a positive history for depression and who were sedentary were more likely to display depressive symptoms ($P = 0.013$ and $P = 0.032$, respectively). In conclusion, the SNP *rs948854* in the GAL gene seems to be involved in the modulation of depressive state, especially in individuals with GG genotype.

1. Introduction

It is well established that several mood disorders could have precursors in early life (Costello et al., 2003; Egger and Angold, 2006). Depression is probably the best known and most frequent mood disorder in adolescence (Costello et al., 2003; Egger and Angold, 2006; Sihvola et al., 2007; Chaplin et al., 2009). In fact, this stage of development is marked by profound changes, not only physical but also psychological, since adolescents do not have full capacity to observe and verbalize your feelings (Costello et al., 2003; Egger and Angold, 2006; Sihvola et al., 2007; Chaplin et al., 2009).

Although several factors (e.g., genetic, social, and environmental conditions) can contribute to the development of depression, the pathophysiological mechanisms are far from clear. Recent studies have demonstrated a significant correlation between the neuropeptide galanin (GAL) and depression (Kuteeva et al., 2008; Unschuld et al., 2010; Juhasz et al., 2014; Wang et al., 2014). Changes in the galaninergic system are correlated with anxiety disorders, depression, and stress. The level of circulating GAL was increased in patients with major depressive disorder (Wang et al., 2014). Particularly in women, there was a positive correlation between plasma GAL levels and severity of the depressive symptoms (Wang et al., 2014). A recent study reported a significant correlation between the presence of single nucleotide polymorphism (SNP) in the GAL gene and its receptors with symptoms of depression and anxiety, only in individuals exposed to stressors like childhood adversities and/or recent adversities in life (Juhasz et al., 2014).

GAL is distributed throughout the brain including several regions implicated in the pathogenesis of depression (Kuteeva et al., 2008; Unschuld et al., 2010; Juhasz et al., 2014; Wang et al., 2014; Lang et al., 2015). However, it should be considered that the effects of GAL will depend on the anatomical area of the brain where its three G-protein coupled receptors (GALR1, GALR2 and GALR3) are expressed, since the activation of these receptors may produce different physiological

* Corresponding author at: Division of Nephrology, School of Medicine, Federal University of São Paulo, Rua Botucatu, 862, 5° floor, - São Paulo, SP 04023-062, Brazil.
E-mail address: maria.franco@unifesp.br (M. do Carmo Franco).

http://dx.doi.org/10.1016/j.yhbeh.2017.10.001
Received 30 April 2017; Received in revised form 1 October 2017; Accepted 3 October 2017

actions (Unschuld et al., 2008; Wang et al., 2014). It is known that GALR1 is highly expressed in hypothalamus, locus coeruleus, ventral hippocampus and nucleus accumbens, whereas GALR2 is expressed in limbic system and hypothalamus (Kuteeva et al., 2008; Lang et al., 2015). On the other hand, GALR3 is low expressed in the brain with more abundance in the hypothalamic-pituitary axis (Lang et al., 2015). This extensive distribution of GAL could promote a wide variety of effects on the brain and behavioral maturation during adolescence. It is known that adolescent brain remains in its active state of maturation, growth, reorganization, and pruning (Arain et al., 2013). Given the brain changes occurring during puberty, and the previous knowledge about GAL distribution and its effects on depression and behavior, this study aimed to evaluate whether genetic variations in the genes encoding GAL and its receptors confer vulnerability to the development of depressive symptoms during adolescence. More specifically, we examined the role of SNP of GAL (*rs4432027*), pre-pro-galanin (PPGAL) (*rs948854*), GAL receptor type 1 (GALR1) (*rs11665337*), and GAL receptor type 2 (GALR2) (*rs8836*). These SNPs were selected from the current literature based on association with depression or mood disorders (Unschuld et al., 2008; Juhasz et al., 2014).

2. Material and methods

This study was carried out in the *Adolescent Healthcare Center*, an outpatient clinic affiliated with Federal University of São Paulo (São Paulo, SP, Brazil), which delivers comprehensive and integrated medical and mental health services to young people aged 10 to 18 years. The present study group comprised 112 adolescents (male/female ratio - 47/65) who were evaluated between September 2013 and April 2014. During this period, we evaluated 200 adolescents who were having their first appointment in the *Adolescent Healthcare Center*. The selection to participate in the present study was based on clinical and psychological evaluations. Detailed history and health information were collected by an experienced pediatrician. Subsequently, all individuals who already had a previous diagnosis of depression, anxiety, schizophrenia, attention-deficit/hyperactivity disorder (ADHD), and bipolar syndrome were excluded. None of the adolescents enrolled in our study were taking specific drugs. Height was measured to the nearest 0.1 cm with a stadiometer and weight to the nearest 0.1 kg with a mechanical medical scale. Body mass index (BMI) was computed using the formula weight (kg)/height (m^2). The same pediatrician, blinded to the clinical data, examined and evaluated all adolescents. The Ethics Committee of the Federal University of São Paulo approved the study (Number: 378.786). All parents and adolescents signed written informed consents/assents.

2.1. Self-report regular physical activity

This questionnaire included two questions regarding the performance and frequency of physical activity. These questions assessed whether the adolescent performed physical activity (yes or no) and the frequency of regular physical activity (Floriani and Kennedy, 2007).

2.2. Children's Depression Inventory (CDI)

The CDI is a self-report measure of depression in children and adolescents elaborated by Kovacs (Kovacs, 1985) that was adapted for use in Brazil (Barbosa et al., 1996). The CDI is a screening tool that can identify children who are at risk of a depressive disorder. All 27 items are rated on a 0 to 2 scale, wherein higher scores indicated more depressive symptoms. The interviews were conducted by a trained pediatrician. All participants completed the CDI in a private room. When necessary, the pediatrician read the questions before the participants, and explained those words/questions that were hard to understand, leaving them free to choose the option they considered the most appropriate. Incidence of depressive symptoms was considered when CDI scores were ≥ 17 points (Kovacs, 1985; Kazdin, 1989).

2.3. Genomic DNA isolation

Buccal cells were collected with cytobrush and stored in − 80 °C. Within 12–24 h after this procedure, DNA was extracted with the QIAamp DNA Blood Mini Kit (Qiagen Inc., CA, USA) according to the manufacturer's protocol. DNA integrity was determined by gel electrophoresis, and DNA yield and quality were assessed by spectrophotometric analysis (NanoDrop2000 UV–Vis Spectrophotometer, Thermo Scientific, USA).

2.4. Amplification and SNPs genotype

Genotyping was performed with TaqMan® SNP Genotyping (Applied Biosystems. Inc., Foster City, CA, USA) for GAL (*rs4432027* - Assay ID: C_1514992_10); PAIL (*rs948854* - Assay ID: C_8760681_10); GALR1 (*rs11665337* - Assay ID: C_1749064_10), and GALR2 (*rs8836* - Assay ID: C_1331486_1_). DNA was amplified separately for each SNP by real time polymerase chain reaction (PCR; Rotor Gene 6000, Qiagen, USA) with 1.25 μl of TaqMan SNP Genotyping Assay (20 ×), 12.5 μl Master Mix (2 ×), and 15 ng of the DNA in a final volume of 25 μl. The cycling conditions were as follows: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min.

2.5. Statistical analysis

All continuous variables were examined for normality with the Shapiro-Wilk test. Categorical variables were presented as percentage and compared with the χ^2 test. Continuous variables were presented as mean ± standard deviation (SD) and median (minimum–maximum) according data distribution. Comparisons of CDI score medians between genotypes were made with the Kruskal-Wallis test, followed by the Dunn-Bonferroni post-hoc test. Haploview software was used to analyze the SNPs and to determine their Hardy-Weinberg (HW) equilibrium status. A binomial logistic regression analysis was performed to assess the association between depression (CDI score ≥ 17; dependent variable) and several independent predictor variables (i.e., gender, age, maternal history of depression, physical activity practice, and genotypes of rs948854 in the GAL gene). The descriptions of this analysis were made by use of odds ratio (OR) and 95% confidence intervals (95% CI). The statistical power of our sample was calculated for the Kruskall-Wallis test for CDI score (80%) and for each SNP by PASS Sample Size Software (NCSS, Statistical Software). The power for two SNPs was higher than 70%, including GAL *rs948854* (75%) and *rs11665337* in the GALR1 receptor (73%). The powers for GAL *rs4432027* and GALR2 receptor *rs8836* were 61% and 60%, respectively. All statistical comparisons were made with SPSS version 21.0 for Windows (IBM Corporation, USA).

3. Results

The study was conducted on 112 adolescents (male/female ratio - 47/65) with a mean age of 14.0 ± 2.1 years. Out of the 112 participants, 100 (89.3%) lived with their families, 94 (85%) reported pubertal stage, 32 (28.6%) failed a grade in school, and 49 (43.8%) practiced physical activity at least twice a week. The mean BMI of the study population was 25.1 ± 6.9 kg/m^2. Furthermore, 34% of adolescents were obese, and 6.4% had morbid obesity (Table 1). In addition, 34 (30.4%) of the participants had depression (CDI score ≥ 17; Table 1). Female adolescents were more likely than male adolescents to be depressive (38.5% vs. 19.1%, $P = 0.028$).

We evaluated four SNPs in the genes encoding GAL and GALR1/GALR2 receptors in these adolescents, and determined the association of these SNPs with gender, current nutritional status, and CDI score. The distribution of genotypes of the four SNPs (*rs4432027*; *rs948854*;