**-759C>T Polymorphism of the HTR2C Gene is Associated with Second Generation Antipsychotic-Induced Weight Gain in Female Patients with Schizophrenia**

**Abstract**

**Introduction:** The *HTR2C* gene is an important candidate in pharmacogenetic studies of antipsychotic-induced weight gain (AIWG). However, inconsistent results have been obtained. The present study investigated the association between -759C>T, functional polymorphism of the *HTR2C* receptor, and AIWG.

**Methods:** A prospective cohort of 48 female inpatients with schizophrenia and related illness treated according to normal clinical practice with second generation antipsychotics (SGAs) risperidone, clozapine, quetiapine, and olanzapine were evaluated. Patients were weighted at admission and again at 6 weeks of hospitalization. Weight gain was defined as an increase ≥ 7% of baseline weight. The association between polymorphisms *HTR2C* and weight gain was evaluated. Multiple logistic regression was run to determine potential confounders.

**Results:** Patients with the T allele at position -759 (TT or CT) gained less weight as compared to patients who did not have the allele. This association was not affected by possible confounding factors such as age, baseline BMI, and prior psychopharmacological treatment.

**Discussion:** The T allele at position -759 protects against AIWG in female patients with schizophrenia.
tion and weight gain among patients diagnosed with schizophrenia, evidencing conflicting results. Some of these studies have found that individuals with the T allele of the HTR2C have less weight gain during treatment with SGAs [14–22]; others have failed to reproduce this result [23–26], while others found the opposite result [27]. There are several possible causes for this discrepancy, such as previous antipsychotic treatment, patient’s ethnicity, and duration of SGA trial. It is noteworthy that in addition to the -759C>T polymorphism of the HTR2C gene other polymorphisms have been involved in genetic studies related to metabolic disturbances and antipsychotics [28–30].

The aim of the present study was to examine the possible association between the -759C>T polymorphism of the HTR2C gene and AIWG among female patients diagnosed with schizophrenia. It was hypothesized that the variant -759T alleles would evidence a protective effect against AIWG.

Methods

Study design

A prospective cohort of inpatients treated according to normal clinical practice with SGAs was evaluated to assess the possible association between a HTR2C receptor polymorphism and AIWG.

Participants

The sample consisted of 48 Caucasian women diagnosed with schizophrenia or related illness admitted at the Emergency Service of the “Dr. Braulio A. Moyano” Neuropsychiatric Women Hospital in Buenos Aires City, Argentina.

Measures and assessment

The present study was approved by the Ethics Committee of the Institution. Following hospital admission, all potential patients were given a complete description of the study and invited to participate. All participants and their legal representatives gave informed written consent for participation. Inclusion criteria included meeting the Diagnostic and Statistical Manual-Fourth Edition Text Revision (DSM-IV-TR) criteria for schizophrenia, brief psychotic disorder, schizophreniform disorder, or delusional disorder and receiving treatment with SGA having high (olanzapine and clozapine) or moderate (risperidone, or delusional disorder and receiving treatment with SGA schizophrenia, brief psychotic disorder, schizophreniform disorder, or delusional disorder. Also, a list of questions regarding demographic and clinical variables was obtained. At admission, (baseline) weight, height, and calculation of body mass index (BMI, defined as weight [kg]/height² [m²]), using standardized procedures, were obtained. During the study period, all patients received the same dietetically balanced hospital meals. At the sixth week of treatment (trial’s end), measurement of weight and BMI calculation were obtained.

At the last measurement, the researcher obtained a sample of the patients’ peripheral blood, via the index finger prick with a lancet, to determine the HTR2C polymorphism. The peripheral blood drops were placed on Whatman FTA Classic Cards (Cat. no. WB120205; Whatman International Ltd, Piscataway, NJ, USA). Patients then continued treatment according to the physician’s clinical judgment. Samples were transported to the Laboratory of Molecular Microbiology, National University of Quilmes, Argentina, where the genetic analysis was performed.

Genetic analysis

Following the 6-week period, all genotyping procedures were conducted by a researcher who was unaware of the clinical status of the patients. We amplified a 252 bp fragment of the HTR2C gene regulatory region from genomic DNA by polymerase chain reaction (PCR), as described by others [31]. The polymorphism analysis of the PCR product was performed by DNA sequencing using the BigDye terminator methodology with an ABI 3130xl Genetic Analyzer (Applied Biosystems/PerkinElmer, Foster City, CA, USA). Nucleotide sequence editing and analyses were performed using BioEdit v7.0.9 (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and ClustalX2 v2.1 (http://www.clustal.org/clustal2/).

Statistical analysis

Categorical measures were reported as frequency or percentage and compared by use of contingency tables (χ²). Continuous measures were reported as mean ± standard deviation (SD) and compared by ANOVA methods (t-test) or Wilcoxon rank-sum test (Mann-Whitney U-statistic) for non-normally distributed continuous data.

Given the hypotheses were that the variant -759T allele would have a protective effect against AIWG, the CT heterozygous and TT homozygous (for -759C>T) were combined for the analysis. To make our results comparable with other studies we classified patients as weight gainers (≥ 7% of baseline weight) and non-weight gainers (< 7% of baseline weight).

Multiple logistic regression modeling was used to evaluate factors significantly associated with AIWG. Specifically, the independent variables age, prior psychopharmacological treatment, and baseline BMI were included at stepwise. Adjusted slope (ß) coefficients and their 95% CI from logistic regression modeling were reported, with significance evaluated using Wald tests. Statistical significance was set at 2-tailed p < 0.05. Analyses were done via STATA.12 (StataCorp, College Station, TX, USA) commercial statistical software.

Results

The current study included a total of 48 inpatient women treated with SGAs. The average age of the female sample was 34.48 years (SD = 13.13), and almost 2-thirds reported no previous psy-
After 6 weeks of treatment with SGAs, the average weight gain of the T allele (n = 11, 23%) was 2.23 ± 0.40 kg. Those patients with the -759T allele gained weight as compared to patients who did not have the allele (χ² = 4.91, p = 0.03).

Because the relationship between polymorphism and weight gain can be influenced by certain factors such as age, baseline BMI, and the presence of previous drug treatments, a multiple logistic regression model was used to analyze each of these possible effects. There were no significant effects of these factors within the relation between genetic polymorphisms and AIWG (Table 2).

### Discussion

The present study provides further evidence of the protective role of the -759T variant allele of the -759C > T polymorphism (rs3813929) of the HTR2C gene against AIWG in female patients with schizophrenia.

Weight gain is a common and important side effect associated with the use of SGAs; however, mechanisms underlying this side effect are not fully understood [32]. Serotonin is thought to play a key role in regulation of feeding behavior and satiety signaling. Indeed, serotonin receptors are considered an important candidate gene in pharmacogenetic studies of AIWG. The 5-HT₂C receptor, encoded by the HTR2C gene, mediates at least in part the metabolic side effects of SGAs and is considered one of the most promising targets [33].

The gene encoded in the 5-HT₂C receptor is located in chromosome Xq24 and presents numerous polymorphisms. One of these polymorphisms (-759C > T) located in the promoter region is known to regulate gene expression [11, 30]. The modifications in the expression of the 5-HT₂C receptor, resulting from genetic variation in HTR2C, may increase the levels of circulating leptin, resulting in greater weight gain. Variants that decrease expression of the receptor may be protective of weight gain induced by SGAs [13, 34].

This polymorphism has been targeted in association studies between treatment with SGAs and weight gain, resulting in variable results. Yuan et al. (2000) were the first to report that the frequency of the variant -759T was greater in non-obese individuals, suggesting that the T allele operates as a protector of obesity in non-schizophrenic patients [31]. After that, Reynolds (2002) suggested a protective effect of the variant -759T allele against antipsychotic-induced weight gain in Chinese Han first episode schizophrenia patients receiving risperidone and chlorpromazine [35].

The subsequent pharmacogenetic studies that
investigated this polymorphism reported inconsistent results. Some of them replicated these results analyzing patients treated with clozapine, olanzapine, or risperidone [14, 17–22]. The current study supports the protective role of the -759T allele against AIWG in schizophrenic patients and provides more knowledge of this effect in female gender. Others studies have not found a positive association between this polymorphism and AIWG [23, 24, 26, 27, 36]. Many factors may explain these differences, such as previous antipsychotic treatment, patient’s ethnicity, and duration of SGA trial. Previous antipsychotic treatment seems to be the strongest factor that accounts for these discrepancies. Importantly, most of the studies with negative findings were performed with patients undergoing previous antipsychotic treatment [23–27]. Correspondingly, a meta-analysis [37], recently re-analyzed [38], demonstrated the overall risk of weight gain in patients without the T allele polymorphism was 2.7 times more likely than those with T allele, but when stratifying these results, an OR of 1.6 was observed in chronic schizophrenic patients in comparison with an OR of 5.4 in first episode patients. Altogether, this information means that the effect size of the association could be larger in first episode patients. The current study sample consisted of patients most of whom had no previous treatments, which may have influenced the positive association found. Furthermore, ethnicity may also account for these differences. For example, most of the previous studies included samples of European-American patients, where a significant association was detected; however, these findings were not replicated among some Asian populations (Korea and Japan). The current study included Caucasian patients mostly of European descent, which may account for the positive finding. Finally, duration of the treatment may also play a role in the positive association observed. Tempelman et al. (2005) investigated the association between the -759T allele and body weight gain in a Caucasian Spanish patient sample characterized by a first episode of psychosis at 6 weeks, 3 months, and 9 months, detecting that the most prominent association was found for short-term weight increases (6 weeks) [14]. The short duration (6 weeks) of treatment yielded by the current inpatient study design may also account for the positive findings of the impact of the -759T polymorphism on AIWG.

Since the HTR2C gene is located on the X chromosome, the association of the -759T allele with AIWG has been thought to be dependent on sex. For example, Reynolds et al. (2003) found that the association was strongest in male patients and not apparent in female patients, suggesting that the protective effect of the allele was dependent on gender; however, their sample included only 11 women, which does not allow for definitive conclusions about this issue [16]. The majority of these studies published were performed in mixed samples with a low number of women, indicating an underpowered sample to detect sex differences. This was confirmed by Kuzman et al. (2008), who performed an association study with a female sample and concluded that the -759C>T polymorphism of the HTR2C gene does not play a significant role in susceptibility to weight gain in women [36]. Our results differ from those of Kuzman et al. probably given the differences between the durations of the studies (4 months in theirs vs. 6 weeks in ours).

There are several potential confounders found in the relation between the -759T allele and AIWG, such as age, initial BMI, and previous antipsychotic treatment. In the present study, we used the logistic regression in order to establish the role of these factors. Based on the results of the regression, we can conclude that the protective effect on weight gain conferred by the T allele was independent of these factors.

The present study has relevance for the development of predictive tests to help clinicians to identify which patients are more susceptible to AIWG.

**Limitations**

The present study has some limitations that should be considered. First, based on the experimental design, there are some variables related to weight gain that were unable to be controlled for, such as diet and physical activity. However, only inpatients were enrolled in the study; all followed the same diet plan during hospitalization with no free access to food or snacks. Physical activity was also comparable for all patients throughout the study period. Second, the study sample was small; yet, the “Braulio A. Moyano” Hospital is only for women, which allowed us to obtain a female homogenize sample. Given that gender differences are important in AIWG, the results add to the topic. Finally, the design of the study is observational (prospective cohort), and therefore, neither the type nor the dose of SGA was defined experimentally. The modest number of patients with different types of SGAs limited the statistical power to analyze the association for different antipsychotics; however, all SGAs employed are considered to induce weight gain and the doses are within the usual range of doses employed in the clinical practice. Taken together, the results reproduce the same conditions as in a clinical setting.

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**Conflict of Interest**

No author or immediate family member has financial relationships with commercial entities that might appear to represent a potential for conflicts of interest.

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