Relationship between the rs1414334 C/G polymorphism in the HTR2C gene and smoking in patients treated with atypical antipsychotics

Relación entre el polimorfismo rs1414334 C/G del gen HTR2C y tabaquismo en pacientes tratados con antipsicóticos atípicos

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Abstract

An association has been found between the C allele of the rs1414334 polymorphism in the HTR2C gene and the metabolic syndrome in psychiatric patients. However, no study has yet evaluated whether this allele is associated with smoking. To assess this issue, therefore, we performed a cross-sectional study with a sample of 166 adult patients treated with atypical antipsychotics in 2012-2013 in a region of Spain. The primary variable was the presence of the C allele of the rs1414334 polymorphism in the HTR2C gene. Secondary variables were the number of pack-years (number of cigarettes per day x number of smoking years ÷ 20), age, gender, schizophrenia, years since diagnosis, metabolic syndrome criteria and SCORE. A stepwise binary logistic regression model was constructed to determine associations between primary and secondary variables and their area under the ROC curve (AUC) was calculated. Of the total sample, 33 patients (19.9%) had the C allele of the polymorphism analyzed. Mean cigarette consumption was 11.6 pack-years. The multivariate analysis showed the following factors as associated with the polymorphism: higher cigarette consumption, being a woman, and not having abdominal obesity. The AUC was 0.706. An association was found between increased cigarette consumption over the years and the presence of the C allele of the rs1414334 polymorphism in the HTR2C gene.

Keywords: Smoking; Pharmacogenetics; Alleles; Psychiatry.

Resumen

En pacientes psiquiátricos, otros autores han encontrado una asociación entre el alelo C del polimorfismo rs1414334 del gen HTR2C y el síndrome metabólico. Ninguno de ellos ha valorado si este alelo se asocia con el consumo de tabaco, por lo que se decidió realizar un estudio en una región española valorando esta cuestión. Estudio observacional transversal de una muestra de 166 pacientes adultos tratados con antipsicóticos atípicos en 2012-2013. Variable principal: presencia del alelo C del polimorfismo rs1414334 del gen HTR2C. Variables secundarias: número de paquetes-año (número de cigarrillos al día x número de años fumando ÷ 20), edad, sexo, esquizofrenia, años con el diagnóstico, criterios de síndrome metabólico y SCORE. Se construyó un modelo de regresión logística binaria por pasos para determinar asociaciones entre la variable principal y las variables secundarias del estudio y se calculó su área bajo la curva ROC (ABC). Del total de la muestra, 33 pacientes presentaron el alelo C del polimorfismo analizado (19,9%). El consumo de tabaco medio fue de 11,6 paquetes-año. El modelo multivariante arrojó los siguientes factores asociados al polimorfismo: mayor consumo tabáquico, ser mujer y no tener obesidad abdominal. El ABC fue de 0,706. Se ha encontrado asociación entre un mayor consumo de tabaco a lo largo de los años y la presencia del alelo C del polimorfismo rs1414334 del gen HTR2C. Se requiere otros estudios que corroboren nuestros resultados.

Palabras clave: Tabaquismo; Farmacogenética; Alelos; Psiquiatría.

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The HTR2C receptor has various functions, including appetite regulation and glucose homeostasis. It is also associated with the use of psychoactive substances, as well as being linked to diseases such as cyclothymic disorder, suicide or premature ejaculation (The Weizmann Institute of Science Crown Human Genome Centre, 2014).

The existing literature on the *HTR2C* rs1414334 polymorphism focuses on the relationship between the presence of the C allele of this polymorphism in psychiatric patients receiving antipsychotics and the possible link with the development of the metabolic syndrome, assessing each of the components of this syndrome individually as well as altogether. The results of these studies generally show an association between the C allele of this polymorphism and the metabolic syndrome (Gregoor et al., 2010; Hoekstra et al., 2010; Houston et al., 2012; Klemettilä et al., 2010; Ma et al., 2014; Mulder et al., 2007a; Mulder et al., 2007b; Mulder et al., 2009; Rico-Gomis et al., 2016; Risselada et al., 2012). In other words, these patients have an increased cardiovascular risk. Moreover, we must bear in mind that there is another major risk factor in the development of cardiovascular disease that is not contemplated in the criteria for the metabolic syndrome, which is smoking (Conroy et al., 2003; Wilson et al., 1998). None of the previous studies have evaluated whether patients with this allele have an association with smoking (Gregoor et al., 2010; Hoekstra et al., 2010; Houston et al., 2012; Klemettilä et al., 2010; Ma et al., 2014; Mulder et al., 2007a; Mulder et al., 2007b; Mulder et al., 2009; Rico-Gomis et al., 2016; Risselada et al., 2012). This is an important issue because animal models have shown that the *HTR2C* receptor modulates nicotine addiction in mice and that stimulation of the *HTR2C* receptors reduces dopamine function at the mesolimbic level (Guy and Fletcher, 2014), decreasing the stimulating effects of nicotine (Fletcher, Lê & Higgins, 2008). Given the lack of studies (Gregoor et al., 2010; Hoekstra et al., 2010; Houston et al., 2012; Klemettilä et al., 2010; Ma et al., 2014; Mulder et al., 2007a; Mulder et al., 2007b; Mulder et al., 2009; Rico-Gomis et al., 2016; Risselada et al., 2012), the possible association between smoking and this receptor, and that tobacco dependence has been found with other genes (Barrot, Sánchez, Abellana, Ortega & Gené, 2013; Fletcher et al., 2008; Guy and Fletcher, 2014; Saccone et al., 2007; Verde et al., 2011; Walton, Johnstone, Munafò, Neville & Griffiths, 2001), we decided to conduct a study to evaluate the possible association between smoking and the C allele of the *HTR2C* rs1414334 polymorphism in psychiatric patients with atypical antipsychotics. The results will help to provide scientific evidence on consumption of toxic substances in these patients and to improve the clinical guidelines for dual pathology and thus improve the success of cessation treatments in this population (San et al., 2016).

Methods

Ethical considerations

Before participating in the study all patients were properly informed and signed an informed consent prior to their inclusion in the study. The study was approved by the Ethics and Research Committee of the General University Hospital of Elche on November 20, 2012. All the procedures followed were in accordance with the Helsinki Declaration, as revised in 2004.

Study population

The study population comprised psychiatric patients treated with atypical antipsychotics in the Department of Health 20 (General University Hospital of Elche). This department is located in the Valencian Community, which is a Mediterranean region of approximately 5 million inhabitants, located in southeast Spain. In this department, health coverage for psychiatric patients is universal and free.

Study design and participants

This was a cross-sectional study of a sample of all adult patients attending the mental health services of Health Department 20 between December 2012 and June 2013 who agreed to participate in the study voluntarily. Inclusion criteria required that patients had to be diagnosed by their psychiatrist with at least one of these conditions: 1) schizophrenia; 2) schizophreniform disorder; 3) schizoaffective disorder; 4) other psychotic disorders; 5) bipolar disorder with treatment that included continuous antipsychotic medication (American Psychiatric Association, 2000). Additionally, all patients had to be on treatment with atypical antipsychotics (clozapine, olanzapine, risperidone, quetiapine, aripiprazole, ziprasidone, amisulpride, asenapine and paliperidone), for at least three months. Patients taking this type of drug were chosen because the HTR2C receptor is involved in its mechanism of action and this gene is in turn associated with the use of psychoactive substances (The Weizmann Institute of Science Crown Human Genome Centre, 2014).

Variables and measurements

The primary study variable was the presence of the C allele of the rs1414334 polymorphism in the *HTR2C* gene. A venous blood sample was obtained from each selected patient (EDTA tube). Genomic DNA was isolated and purified from buffy coat samples using the semi-automated QIAcube system (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Extracted DNA was used for genotyping. Analyses of the polymorphism (rs1414334 C/G) were performed using a Taqman allelic discrimination assay (C_7455701_10, Applied Biosystems, Madrid, Spain) on a real-time PCR apparatus (Applied Biosystems 7300) (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1414334).

The secondary variables were: age (years), gender, schizophrenia (yes/no), years since diagnosis of the disorder, number of pack-years (number of cigarettes per day × number of years of smoking ÷ 20), metabolic syndrome criteria (abdominal obesity, hypertriglyceridemia, hypertension, abnormal HDL-c and insulin resistance) and SCORE (%) (Conroy et al., 2003; Grundy et al., 2004). The main diagnosis and its duration, age and gender were obtained from the patients' medical records. The number of cigarettes and the time each subject had smoked was obtained by personal interview. The metabolic syndrome criteria were obtained through measurements of blood pressure (systolic and diastolic), blood tests (total cholesterol, HDL-c, triglycerides and fasting glucose) and abdominal circumference. These measurements were performed according to the applicable clinical guidelines (American Diabetes Association, 2012; National Institutes of Health, 1998; Mancia et al., 2007; National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2002). Finally, the SCORE was calculated using systolic blood pressure, age, gender, atherogenic index (total cholesterol ÷ HDL-c) and smoking as a binary variable (smoking or no smoking).

Sample size

Given that an a priori calculation of sample size was not performed, we calculated the statistical power of the sample during the study period: 166 patients, of whom 33 had the C allele of the polymorphism. To estimate an area under the ROC curve (AUC) different from 0.5, assuming an AUC of 0.70 and a confidence level of 95%, a power of 94.43% was obtained (Hanley & McNeil, 1982).

Statistical methods

Qualitative variables were described by absolute and relative frequencies, and quantitative variables using means and standard deviations. Raw odds ratios (ORs) were calculated to determine possible associations between the secondary variables and our primary variable. To obtain the adjusted ORs, a stepwise binary logistic regression model was constructed, considering that we could only enter a maximum of 3 variables (one for every 10 events). The steps were to obtain all possible combinations of the explanatory variables (231) and to calculate the AUC of the resulting model with that combination, keeping the combination with the highest AUC (the model with the greatest discriminatory power) (Azrak et al., 2015; Gutiérrez-Gómez et al., 2015; López-Bru, Palazón-Bru, Folgado-de la Rosa & Gil-Guillén, 2015; Ramírez-Prado et al., 2015). The type I error was set at 5% and for each relevant parameter its associated confidence interval (CI) was calculated. The statistical packages used were R 2.13.2. and IBM SPSS Statistics 19.

Results

Of a total of 166 patients treated with atypical antipsychotics, 33 had the C allele of the polymorphism analyzed (19.9%, 95% CI: 13.8-26.0%). Table 1 shows the descriptive and analytical characteristics of our patient sample. We highlight a mean age of 43.1 years, 55.4% with schizophrenia and mean number of years with the primary diagnosis of 14.9. Tobacco consumption was 11.6 pack-years on average. Finally, the metabolic syndrome criteria were highly prevalent (abdominal obesity, 66.3%; hypertriglyce-

Table 1. *Descriptive and analytical characteristics of the patients diagnosed with psychiatric disorders in a Spanish region: analysis of the rs1414334 C polymorphism in the HTR2C gene.*

Variable	**Total** n=166 n(%)/x±s	**Raw OR** (95% CI)	**p-value**	**Adj. OR** (95% CI)	**p-value**
Age (years)	43.1±11.5	1.02(0.98-1.05)	0.318	N/M	N/M
Gender, female	67(40.4)	2.40(1.11-5.22)	0.027	4.31(1.67-11.17)	0.003
Schizophrenia	92(55.4)	0.52(0.24-1.12)	0.094	N/M	N/M
Years with the disorder	14.9±9.6	0.99(0.95-1.03)	0.486	N/M	N/M
Number of pack-years	11.6±17.5	1.02(1.00-1.04)	0.057	1.03(1.01-1.05)	0.008
Abdominal obesity[a]	110(66.3)	0.70(0.31-1.58)	0.399	0.39(0.15-1.01)	0.053
Hypertriglyceridemia[a]	64(38.6)	0.53(0.23-1.23)	0.130	N/M	N/M
Hypertension[a]	58(34.9)	0.76(0.33-1.73)	0.511	N/M	N/M
Abnormal HDL-c[a]	55(33.1)	0.85(0.37-1.94)	0.698	N/M	N/M
Insulin resistance[a]	31(18.7)	0.54(0.18-1.67)	0.261	N/M	N/M
SCORE (%)	0.80±1.51	1.20(0.96-1.50)	0.107	N/M	N/M

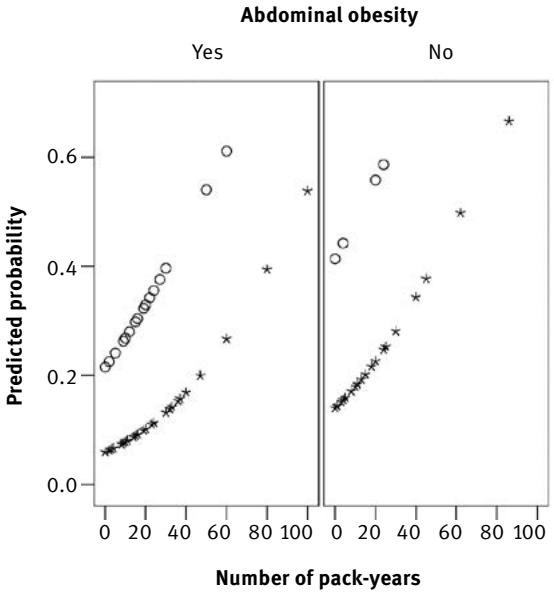
Nota. n(%), absolute frequency (relative frequency); x±s, mean ± standard deviation; Adj. OR, adjusted odds ratio; Raw OR, raw odds ratio; CI, confidence interval; N/M, not in the model. Goodness-of-fit of the multivariate model: X^2=14.80, p=0.002; area under the ROC curve=0.706 (95% CI: 0.603-0.810, p<0.001). [a] Criteria for Clinical Diagnosis of the Metabolic Syndrome (ATP III).

ridemia, 38.6%; hypertension, 34.9%; abnormal HDL-c, 33.1%; insulin resistance, 18.7%).

Univariate analysis of the associated factors (Table 1) showed a statistically significant association ($p<0.05$) between the C allele of the polymorphism and being a woman and greater tobacco consumption, while not having schizophrenia remained close to statistical significance ($0.05< p<0.10$). After adjusting with the best combination of factors (AUC=0.706), we found that higher tobacco consumption, being a woman and not having abdominal obesity were associated with the allele analyzed. The model that included these factors was highly significant ($p=0.002$).

Figure 1 shows the predicted probabilities of the presence of the C allele of our polymorphism. These probabilities increased as the patient's tobacco consumption increased. Moreover, being a woman and not having abdominal obesity indicated a greater likelihood of having this allele.

Figure 1. Predicted probabilities of the presence of the C allele of the HTR2C rs1414334 polymorphism according to specific variables in the multivariate model.



Note. Gender: * Male; O Female.
Goodness of fit: p=0.002.

Discussion

Summary

In an innovative way, this study identified the association between tobacco consumption measured in pack-years and the presence of the C allele of the rs1414334 polymorphism in the *HTR2C* gene. This association was direct; that is, the higher the patient's tobacco consumption, the higher the probability of having this allele. Moreover, it was found that being a woman and not having abdominal obesity were associated with having this allele.

Strengths and limitations of the study

The main strength of this work is the research question addressed. We found no studies determining the association between smoking and the C allele of the polymorphism studied, such that our results are novel. Furthermore, all possible combinations of variables were tested to obtain the best multivariate model and the power of the sample was near 95%, when most studies use powers of 80% and 90%.

To minimize selection bias, patient data were collected over a specific period of time. Regarding information bias, the data were carefully and exhaustively collected by the research team. Finally, confounding bias was minimized using the best combination of a total of 231.

Our finding is probably related to the X-bound character of HTR2C (i.e., men are hemizygotes) and loss of statistical power. In addition, some second-generation antipsychotics, including asenapine, clozapine, olanzapine and sertindole, are relatively potent 5-HT receptor antagonists and others, including amisulpride, asenapine, clozapine, amisulpride, lurasidone and risperidone, have high affinity for 5-HT receptors. The effects of these second-generation antipsychotics may distort a possible relationship with genetic variations in receptor activity. Therefore, the relationship between the 5-HT2C polymorphism and smoking is possibly due to camouflage by receptor binding caused by the current drug treatment. On the other hand, smoking was measured by interview, instead of expired CO.

Comparison with the existing literature

When assessing the association between tobacco consumption and the allele examined, we found no studies with which to compare our results because previous studies did not consider smoking among their variables (Gregoor et al., 2010; Hoekstra et al., 2010; Houston et al., 2012; Klemettilä et al., 2010; Ma et al., 2014; Mulder et al., 2007a; Mulder et al., 2007b; Mulder et al., 2009; Rico-Gomis et al., 2016; Risselada et al., 2012). We believe that this association may be related to the role of the *HTR2C* receptor in modulating nicotine in the brain, since similar behavior has been seen in animal models using mice (Fletcher et al., 2008; Guy & Fletcher, 2014). However, this should be corroborated through genetic and experimental studies.

With respect to the other associations found, gender is consistent, since this gene is located on chromosome X. Finally, the association between abdominal obesity and this allele (not significant in our study) is not clear in the previous two studies published on the subject, since one of them found a direct association and the other found no association between these two variables (OR close to 1) (Mulder et al., 2007a; Risselada et al., 2012).

The C allele was present in approximately one in every five patients treated with atypical antipsychotics, which is

similar to the results found by others (Hoekstra et al., 2010; Mulder et al., 2007a; Mulder et al., 2007b; Mulder et al., 2009; Risselada et al., 2012).

Implications to research and practice

Given that tobacco consumption was associated with the C allele of the *HTR2C* rs1414334 polymorphism, if these results are confirmed by other studies, we will be able to determine which patients are most likely to have high tobacco consumption and carry out early intervention to attempt to partially decrease or completely eliminate the patient's smoking habit. In turn, this would reduce their cardiovascular risk (Prescott, Hippe, Schnohr, Hein, y Vestbo, 1998), which is higher than in the general population (McEvoy et al., 2005; Newcomer & Hennekens, 2007; Saha, Chant & McGrath, 2007). According to the results obtained, a study could be carried out analyzing the C allele in a sample with a high number of subjects, comparing the smoking population with the nonsmoking population, including men and women, and stratifying by psychiatric history and by metabolic syndrome. Additionally, variables related to smoking, such as cessation attempts, success/failure in discontinuation and drugs used for smoking cessation could be included.

Conclusions

This study found a direct association between increased consumption of tobacco over the years and the presence of the C allele of the rs1414334 polymorphism in the *HTR2C* gene. Since we have found no reports evaluating this association, studies to corroborate our results are needed. If this association is verified, we will know which patients treated with atypical antipsychotics are more likely to have a high tobacco consumption.

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Conflict of interest statement

Nothing to declare.

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