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Lack of association between the $5HT_{2A}$ receptor polymorphism (T102C) and unipolar affective disorder in a multicentric European study

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Abstract

We report here a case-control association study with T102C polymorphism in the serotonin 2A receptor gene (HTR2A) in patients affected by unipolar affective disorder (UPAD) and in controls. A total of 284 subjects were genotyped (142 UPAD and 142 controls). All subjects were interviewed using standard diagnostic interviews and matched. A homogenous population of unipolar patients with suicidal attempt was identified. Conditional logistic regression was applied. No association of the HTR2A polymorphism was found in the overall sample of 142 UPAD-control pairs regarding allele and genotype frequencies ($P = 0.36$ and $P = 0.52$ respectively) and homo-heterozygote distributions ($P = 0.91$). This study confirms, in a multicentric European sample, the earlier observations that the T102C HTR2A polymorphism is not associated with UPAD. Nevertheless, a type 2 statistical error cannot be excluded. Therefore, to exclude the implication of HTR2A in UPAD, this result must be replicated in larger samples and in other populations using the transmission disequilibrium test and different polymorphisms around HTR2A.

Keywords: Serotonin; 5HT2A receptor polymorphism; Unipolar affective disorder; Conditional logistic regression

1. Introduction

Aetiological factors involved in affective disorders have been studied for decades. Besides environmental factors, family, twin, adoption and segregation studies have evidenced the genetic implication in mood disorders (Mendlewicz, 1994). Nevertheless, it becomes more and more obvious that the pattern of inheritance is complex involving genetic and environmental interactions.

The serotonergic system has been implicated in the pathophysiology of mood disorders and in the mechanism of action of many antidepressants. Over the last decade, molecular cloning techniques have revealed numerous types of post-synaptic serotonin (5HT) receptors. Among them, 5HTreceptor type 2A ($5HT_{2A}$) seems to play an important role in the molecular mechanism of unipolar affective disorder (UPAD) (Maes and Meltzer, 1995). The $5HT_{2A}$ receptor gene (HTR2A) is located on chromosome

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13 (13q14–21) and spans 20 kb (Chen et al., 1992). A polymorphism (T102C) has been identified within the coding region of exon 1 (Warren et al., 1993) that does not alter the serine at position 34 (silent mutation). No association was found between this polymorphism and UPAD (Tsai et al., 1999; Frisch et al., 1999; Minov et al., 2001). Only Zhang et al. (1997) found an increased frequency of the T allele at position 102 in 71 patients with depressive disorders. Du and colleagues suggested an association between an increased frequency of allele C and elevated suicidal ideation and behavior (Du et al., 2000). This result was replicated by Arias et al. (2001) in an independent and larger sample. In these studies, an effect of ethnic stratification could not be excluded.

Massat et al. (2000) showed recently no association between the HTR2A polymorphism and bipolar affective disorder in a large sample of 618 unrelated subjects (309 bipolar patients and 309 controls) and concluded that the 5HT2A receptor gene polymorphism is not implicated in bipolar affective disorder.

Regarding treatment response, Serretti et al. (2000) found no association between the HTR2A polymorphism and lithium prophylactic efficacy in bipolar and unipolar patients. Recently, Minov et al. (2001) observed that unipolar depressed patients with one or two C-alleles of the HTR2A polymorphism showed a greater decrease in HAMD-17 and CGI item 1 (severity of illness) after various antidepressant treatments. Cusin et al. (2002) tested a possible effect of HTR2A on the antidepressant activity of fluvoxamine and paroxetine in a sample of unipolar and bipolar patients but concluded that HTR2A did not seem to play a major role in SSRIs antidepressant activity.

From this review, it appears that the T102C polymorphism of the 5HT2A receptor gene is not associated with the unipolar phenotype but may be associated with particular traits related to this disorder (i.e. suicidal ideation and treatment response). However, due to the small sample sizes, no definitive conclusion could be established. The present study describes for the first time, to our knowledge, a matched case-control association study with T102C polymorphism in a multicentric European sample of unipolar patients.

2. Methods

A total of 284 subjects were genotyped (142 UPAD and 142 controls). The patients and controls were recruited within the framework of the Biomedical European Multicentre Collaborative Program (BIOMED 1): European Collaborative Project on Affective Disorders (ECPAD). This network was established within the framework of the European Commission (for objectives and methodology of the project, see Souery et al., 1998). After giving their informed consent, all subjects were using standard diagnostic interviews, i.e. the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-LA; Endicott and Spitzer, 1978) and the Schedule for the clinical Assessment of Neuropsychiatry (SCAN; Wing et al., 1990). One of the two diagnostic interviews was used for all patients and controls recruited for the project. Patients met the diagnosis of UPAD (at least 2 episodes, average number of episodes: 2.1) according to RDC, DSM-III-R and DSM-IV classification systems. Patients and controls were matched to control for potential confounding factors, particularly ethno-geographical origin. A homogenous population of unipolar patients with suicidal attempt was identified. Suicidal attempt was defined as follows: at least one suicidal attempt in personal history. Two analyses were conducted. First, 33 patients with suicide attempt matched with 33 healthy controls were selected. Second, those 33 patients were matched to 33 unipolar patients without suicide attempt in personal history. Previous studies showed an association between violent suicide attempt and genetic markers (Bondy et al., 2000; Turecki et al., 2001). In our population of 33 patients with suicide attempt, 13 had a history of violent suicide attempt. Thus, no statistical analysis was conducted in this subgroup due to the small sample size.

Genomic DNA analysis was isolated from peripheral blood leukocytes using a standard salting out procedure (Miller et al., 1988). The polymorphism in $5HT_{2A}$ was identified by the polymerase chain reaction (PCR) followed by restriction enzyme digestion. Standard PCR was carried out in a 25-μl volume containing 100 ng genomic DNA, 200 μM of each dNTP, 1.25 mM $MgCl_2$, 50 pmol of each primer, and 0.2 units Goldstar DNA polymerase (Eurogentec). Published primer sequences were used (Warren et al., 1993). After an initial denaturation step at 94 °C for 1 min, annealing at 60 °C for 1.5 min, and extension at 72 °C for 2 min. An additional final extension step was performed at 72 °C for 5 min. Twenty microliters of the PCR product were digested overnight at 37 °C with 0.1 units/μl of M*sp*I in a total volume of 25 μl. Digestion products were visualized by ethidium bromide staining after electrophoresis in a 3% agarose gel.

The main advantage of our study was that we applied a conditional logistic regression, which is more appropriate for matched samples as opposed to independent samples. Conditional logistic regression is more outstanding to assess the association between diagnosis and predictor variables and to derive odds ratios and 95% CI intervals after adjustment for potential confounding factors.

Statistical analyses were performed using SPSS. Samples were tested for Hardy–Weinberg equilibrium. Breslow–Day tests were applied before conditional logistic regression.

3. Results

The samples were tested for Hardy–Weinberg equilibrium for UPAD and control populations. No significant