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Role of serotonergic-related systems in suicidal behavior: Data from a case–control association study

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ABSTRACT

Objective: To investigate whether functional polymorphisms directly (HTR2A and SLC6A4 genes) or indirectly (IL-1 gene complex, APOE and ACE genes) related with serotonergic neurotransmission were associated with suicidal behavior.
Subjects and methods: 227 suicide attempters, 686 non-suicidal psychiatric patients, and 420 healthy controls from a homogeneous Spanish Caucasian population were genotyped using standard methods.
Results: There were no differences in genotype frequencies between the three groups. The −1438A/G [χ^2 (df) = 9.80 (2), uncorrected p = 0.007] and IL-1α −889C/T [χ^2 (df) = 8.76 (2), uncorrected p = 0.013] genotype frequencies between impulsive and planned suicide attempts trended toward being different (not significant after Bonferroni correction). Suicide attempts were more often impulsive in the presence of −1438G/G or IL-1α −889C/T or C/C genotypes. There was interaction between the polymorphism 5-HTTLPR and age [LRT (df) = 6.84 (2), p = 0.033] and between the polymorphisms APOE and IL-1RA (86 bp)$_n$ [LRT (df) = 12.21 (4), p = 0.016] in relation to suicide attempt lethality.
Conclusion: These findings further evidence the complexity of the association between genetics and suicidal behavior, the need to study homogenous forms of the behavior and the relevance of impulsive and aggressive traits as endophenotypes for suicidal behavior.

1. Introduction

Since the original description of an increased tendency to more violent and frequent suicide attempts in a subgroup of depressed patients with low levels of the major serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) (Asberg et al., 1976), several studies have reported similar findings and suggested that low 5-HIAA levels are predictive of future suicide attempts and suicide completion (Courtet et al., 2004). These data led Mann et al. (1999) to suggest that the serotonergic system might be related to the diathesis for suicidal behavior and to propose the level of CSF 5-HIAA as a potential endophenotype for genetic studies on suicidal behavior (Mann et al., 2009).

To date, candidate genes for suicidal behavior have been selected largely on the basis of established biological correlates of suicidal behavior and thus have focused primarily on the serotonergic system (Mann et al., 2009). Polymorphisms of serotonin-related genes have been widely studied and related to suicidal (Bondy et al., 2006; Rujescu et al., 2007; Mann et al., 2009) and impulsive behaviors (Baca-Garcia et al., 2005; Nomura et al., 2006). Specifically, association case–control studies have found an association between the short allele (S) of the 5-HTTLPR polymorphism of the serotonin transporter gene (5-HTT gene; SLC6A4) and violent suicidal behavior (Bellivier

Abbreviations: ACE, angiotensin converting enzyme; APOE, apolipoprotein E; bp, base pair; CI, confidence interval; D, deletion; df, degrees of freedom; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders IV; HTR2A, serotonin 2A receptor; 5-HTT, serotonin transporter; I, insertion; IL-1, interleukin-1; IL-1RA, interleukin-1 receptor antagonist; LRT, likelihood ratio test; MDS, Medical Damage Rating Scale; MINI, Mini-International Neuropsychiatric Interview; OR, odds ratio; SD, standard deviation; SIS, Suicidal Intent Scale; SLC6A4, serotonin transporter gene; SPSS, Statistical Package for Social Sciences; VNTR, variable number of tandem repeats; χ^2, Chi-square test.

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et al., 2000; Bondy et al., 2000; Courtet et al., 2001) and a possible effect of the S allele on the number and severity of suicide attempts (Courtet et al., 2004; Gorwood et al., 2000; Saiz et al., 2008a; Wasserman et al., 2007).

Several recent studies have sought to expand the research by identifying other systems that might be indirectly related to low serotonergic activity. Cytokines are a heterogeneous group of polypeptides closely associated with the immune system and the inflammatory process. The link between cytokines and the serotonin system was established by data suggesting that interleukin-1 (IL-1) activates brain serotoninergic systems, increasing brain tryptophan concentrations and serotonin metabolism (Dunn et al., 2005). Zhu et al. (2006) provided evidence that IL-1β can stimulate neuronal serotonin transporter activity, which could decrease extracellular serotonin and be related to the pathophysiology of suicidal behavior and other psychiatric disorders.

Studies have also found evidence of a possible relationship between low cholesterol levels and suicidal behavior (Saiz et al., 1997; Sarchiapone et al., 2000). Apolipoprotein E (APOE) is a major component of lipoproteins and may be involved in cholesterol transport into neurons, thus playing an important role in neuronal growth and in the central nervous system response to injury, particularly in the hippocampal region (Poirier, 1994). The human APOE gene is polymorphic and three common isoforms called ε2, ε3, and ε4 that differ in amino acids at positions 112 and 158 have been described. The ε2 allele has been associated with lower plasma levels of total cholesterol (Liu et al., 1999; Xhignesse et al., 1991).

Finally, the angiotensin converting enzyme (ACE) plays a key role in the renin–angiotensin system because it converts angiotensin I to angiotensin II, an octapeptide thought to affect cognitive function and behavior (Hishimoto et al., 2006) as well as modulating the release and synthesis of serotonin, dopamine, and noradrenaline in the brain (Jenkins et al., 1995). A functional polymorphism at intron 16 of the ACE gene, consisting of an insertion/deletion (I/D) of a 287 base pair (bp) sequence, is associated with blood ACE concentrations. However, I/I individuals have one-third lower serum ACE levels than D/D homozygotes (Tiret et al., 1992). Thus the decreased enzymatic activity associated with the ACE I/I genotype could predispose individuals to suicidal behavior through its negative effect on serotonergic neurotransmission in the brain. On the other hand, an increase in ACE levels associated with the D/D genotype will lead to an increase in substance P and thus of stress and/or anxiety, possibly increasing the risk of suicide (Sparks et al., 2009).

Given that suicidal behavior is a complex multidetermined behavior and the abundant evidence that the serotonergic transmission plays a role in suicidal behavior and the likely gene by gene relationships that might influence this behavior, in this study, we aimed to investigate whether functional polymorphisms directly [HTR2A −1438A/G (rs6311), and SLC6A4 5-HTTLPR and STin2 VNTR] or indirectly [IL-1α −889C/T, IL-1β +3935C/T, IL-1RA $(86\ bp)_n$, APOE, and ACE I/D] related with serotonergic neurotransmission were associated with suicidal behavior. Polymorphisms on the above described genes were chosen based on functionality and clinical relevance.

2. Subjects and methods

2.1. Patient population

The total sample was composed of 227 suicide attempters [mean age (SD) = 36.3 (12.9) years; males: 37%, mean age (SD) = 35.9 (10.6) years; females: 63%, mean age (SD) = 36.5 (14.2) years]. Attempted suicide was defined according to the proposed WHO/EURO definition (World Health Organisation, 1986). Suicide attempters were assessed within 24 h of the attempt using the Suicidal Intent Scale (SIS) (Beck et al., 1974). However, the assessment was impossible in 14 cases due to clinical condition. Lethality was evaluated using the Medical Damage Rating Scale (MDS) (Beck et al., 1975) (unrecorded for 8 patients). Axis I diagnoses were determined in the emergency room using the Mini-International Neuropsychiatric Interview—MINI DSM-IV criteria and clinical records. Only clinical records were used for Axis II diagnoses. Most (88.1%) had at least one Axis I or Axis II diagnosis. Additional prevalent diagnoses were (only main diagnoses are specified): affective disorders (35.7%), schizophrenia and other psychosis (18.5%), anxiety disorders (13.7%), adjustment disorders (7.0%), personality disorders (5.7%), eating disorders (3.5%), and alcohol or drug abuse/dependence (3.1%). No diagnosis was determined in 7 patients (no data in clinical records and impossible to diagnose in the emergency room due to clinical condition). Most attempts (87.2%) were non-violent [i.e., overdose (81.9%), poisoning (4.0%) or gas (1.3%)]. Half (112, 49.3%) had a previous history of attempted suicide.

The psychiatric control group consisted of 686 psychiatric outpatients (42.3% schizophrenia and other psychosis, affective disorders 20.5%, alcohol dependence 23.3%, anxiety disorders 13.9%, diagnosed by the MINI DSM-IV) [mean age (SD) = 41.5 (12.8) years; males: 59.5%, mean age (SD) = 41.1 (12.4) years; females: 40.5%, mean age (SD) = 42.0 (13.5) years].

The healthy control group included 420 unrelated subjects seen consecutively by a general practitioner for an acute, non-serious medical event (e.g., cold, otitis, lumbago, etc.). Only subjects without personal history of psychiatric disorder (MINI DSM-IV and clinical records) or personal or familial history of suicide attempts were included on this group [mean age (SD) = 40.6 (11.3) years; males: 51.4%, mean age (SD) = 41.1 (11.5) years; females: 48.6%, mean age (SD) = 40.2 (11.1) years].

All individuals were of Caucasian Spanish origin and were comparable in sociodemographic profile and geographic origin of their families. All participants provided written informed consent. The study received institutional approval and was subject to and in compliance with national Spanish legislation.

2.2. Genotyping

Genomic DNA was extracted from peripheral white blood cells obtained from each participant according to standard protocols (Miller et al., 1988). All polymorphisms were identified according to previously published methods described elsewhere (Asensi et al., 2003; Cox et al., 1998; Martínez-Barrondo et al., 2005; Rigat et al., 1992; Tarlow et al., 1993; Wenham et al., 1991). Genotype determinations were performed blind to clinical condition. Randomized individuals were re-tested for their genotypes, confirming the pattern reproducibility.

2.3. Data analysis

Observed frequencies were compared with those expected according to the Hardy–Weinberg equilibrium through a Chi-square (χ^2) test.

A cut-point of 6 on the SIS distinguished impulsive from planned suicide attempts (Diaz et al., 2003) whereas MDS score ≥4 defined high lethality (Wasserman et al., 2007).

χ^2 tests were used to assess differences in genotype or allele frequencies across categories of variables such as participant type, suicide attempt impulsivity, etc. A Bonferroni correction coefficient of 8 was adopted in view of the examination of the effects of eight genetic markers (Bland and Altman, 1995). Odds ratios, with exact mid-p 95% confidence intervals, were also calculated as a measure of the difference in the distribution of two alleles between two groups.

Stepwise logistic regression analyses were carried out, with impulsivity and lethality as the dependent variables. The independent variables were sex, age group and the genotypes of the eight