Progress in Neuro-Psychopharmacology & Biological Psychiatry 35 (2011) 1518–1524

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

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

Pilar A. Saiz [a,*], Paz García-Portilla [a], Begoña Paredes [b], Paul Corcoran [c], Celso Arango [d], Blanca Morales [e], Emilio Sotomayor [f], Victoria Alvarez [e], Eliecer Coto [e], Gerardo Flórez [g], María-Teresa Bascaran [a], Manuel Bousoño [a], Julio Bobes [a]

[a] Department of Psychiatry, School of Medicine, University of Oviedo, Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM, Julián Clavería 6–3°, 33006 Oviedo, Spain
[b] Emergency Room, San Agustin Hospital, Camino de Heros 4, 33400 Avilés, Spain
[c] National Suicide Research Foundation, 1 Perrott Avenue, College Road, Cork, Ireland
[d] Unidad de Adolescentes, Department of Psychiatry, Hospital General Universitario Gregorio Marañon, Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM, Ibiza 43, 28009 Madrid, Spain
[e] Laboratory of Molecular Genetics, Central University Hospital of Asturias, Celestino Villamil sn, 33006 Oviedo, Spain
[f] Mental Health Services of Asturias (SESPA), Maestros Martin Garlache sn, 33510 Pola de Siero, Spain
[g] Unidad de Conductas Adictivas, Hospital Santa Maria Nai, CHOU, Ramón Puga 52–56, 32005 Ourense, Spain

ARTICLE INFO

Article history:
Received 3 February 2011
Received in revised form 15 April 2011
Accepted 25 April 2011
Available online 7 May 2011

Keywords:
Angiotensin converting enzyme
Apolipoprotein E
Interleukin-1 gene complex
Serotonin system
Suicidal behavior

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.

* Corresponding author. Tel.: +34 98 510 3552; fax: +34 98 510 3552.
E-mail address: frank@uniovi.es (P.A. Saiz).

0278-5846/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2011.04.011

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

polymorphisms. Rare genotypes were not considered for the regression models. The logistic regression model derived by stepwise selection from all independent variables was re-estimated with only the selected independent variables in order to report the associations based on minimal missing data. Likelihood ratio tests (LRT) were used to examine whether there were interactions between pairs of polymorphisms or between any polymorphism and either sex or age group.

SPSS versions 15.0 and 16.0 (SPSS Inc., Chicago, Illinois, USA) and StatsDirect Statistical Software version 2.5.7 (http://www.statsdirect.com) were used for the statistical analyses. Power calculations were performed using the EPI Info software package version 6 (Center for Disease Control, Atlanta, Georgia, USA). The SHEsis Program (Shi and He, 2005) was used to calculate the linkage disequilibrium (LD) between all pairs of markers, to estimate the frequencies of the SLC6A4 and IL-1 gene complex polymorphism haplotypes in all three groups and to test for between-group differences using a LRT.

The whole sample size was large enough to detect a relative risk of 2.1 with a statistical power of more than 85% and significance of 5% (two-tailed test), assuming a minimum genotype frequency of 10%. However, the statistical power when analyzing differences in impulsivity or lethality was more than 75% to detect a relative risk of 3.

3. Results

The total number of samples analyzed differed among polymorphisms because of sample depletion or repeated assay failure. The three groups (suicide attempters, psychiatric controls, and healthy controls) showed Hardy–Weinberg equilibrium for the analyzed genetic variability with the exception of STin2 VNTR (psychiatric control group, $p = 0.019$) and IL-1RA $(86\ bp)_n$ (suicide attempters, $p = 0.033$; and psychiatric controls, $p = 0.001$). Some degree of LD was found between 5-HTTLPR and STin2 VNTR polymorphisms, and between the three polymorphisms of the IL-1-gene complex, but LD was not complete enough to warrant not genotyping all polymorphisms.

There were no differences in genotype frequencies between suicide attempters, psychiatric patients and healthy controls (Table 1).

3.1. Impulsivity of suicide attempt

Two-thirds (140, 65.7%) of the suicide attempts were considered impulsive, the other third (73, 34.3%) were planned. The proportion of suicide attempts considered impulsive did not vary by age but showed some evidence of being associated with female gender [70.5% vs. 58.0%; χ^2 (df) = 3.44 (1), $p = 0.064$]. There was no evidence of an association between psychiatric diagnosis and impulsivity of the suicide attempt [χ^2 (df) = 3.17 (7), $p = 0.869$].

HTR2A −1438A/G genotype frequencies trended toward being different in impulsive and planned suicide attempts [χ^2 (df) = 9.80 (2), uncorrected $p = 0.007$, corrected $p = 0.056$] (Table 2). Genotype A/A was more common in planned attempts (31.5% vs. 17.9%) whereas genotype G/G was more common in impulsive attempts (32.9% vs. 15.1%). Allele G was associated with impulsive acts [72.5% vs. 58.3%; χ^2 (df) = 9.50 (1), uncorrected $p = 0.002$, corrected $p = 0.016$; OR = 1.88, 95% CI = 1.26–2.83].

IL-1α −889C/T genotype frequency also trended toward being different in impulsive and planned suicide attempts [χ^2 (df) = 8.76 (2), uncorrected $p = 0.013$, corrected $p = 0.104$] though there was no difference in allele frequency [χ^2 (df) = 1.88 (1), $p = 0.170$]. The T/T genotype was more common in planned than impulsive suicide attempts (16.7% vs. 5.0%) whereas C/T was more common in impulsive attempts (40.0% vs. 29.2%).

Suicide attempts were about four times more likely to be impulsive in the presence of −1438G/G relative to A/A whereas an impulsive suicide attempt was four times less likely with IL-1α −889T/T relative to C/T or C/C (Table 3).

Table 1
Genotype frequencies of polymorphisms of the HTR2A, SCL6A4, IL-1α, IL-1β, IL-1RA, AOPE, and ECA genes.

	Suicide attempters[a]	Psychyatric controls[a]	Healthy controls[a]	χ^2 (df)	P
HTR2A −1438A/G	56 (24.7%) A/A	156 (22.7%) A/A	88 (21.0%) A/A	2.72 (4)	.605
	111 (48.9%) A/G	343 (50.0%) A/G	202 (48.1%) A/G		
	60 (26.4%) G/G	187 (27.3%) G/G	130 (31.0%) G/G		
5-HTTLPR[b]	60 (26.5%) L/L	194 (28.3%) L/L	124 (29.5%) L/L	2.44 (4)	.655
	108 (47.8%) L/S	347 (50.6%) L/S	203 (48.3%) L/S		
	58 (25.7%) S/S	145 (21.1%) S/S	93 (22.1%) S/S		
STin2 VNTR	95 (41.9%) 12/12	318 (46.4%) 12/12	174 (41.4%) 12/12	4.15 (6)	.656
	93 (41.0%) 12/10	274 (39.9%) 12/10	177 (42.1%) 12/10		
	36 (15.9%) 10/10	88 (12.8%) 10/10	64 (15.2%) 10/10		
	3 (1.3%) others	6 (0.9%) others	5 (1.2%) others		
IL-1α −889C/T[b]	121 (53.5%) C/C	341 (49.7%) C/C	216 (51.4%) C/C	1.50 (4)	.826
	85 (37.6%) C/T	286 (41.7%) C/T	165 (39.3%) C/T		
	20 (8.8%) T/T	59 (8.6%) T/T	39 (9.3%) T/T		
IL-1β +3953C/T[b]	131 (58.2%) C/C	378 (55.1%) C/C	233 (55.5%) C/C	3.40 (4)	.493
	79 (35.1%) C/T	272 (39.7%) C/T	156 (37.1%) C/T		
	15 (6.7%) T/T	36 (5.2%) T/T	31 (7.4%) T/T		
IL-1RA $(86\ p)_n$[b]	132 (59.5%) A1/A1	384 (56.0%) A1/A1	213 (50.8%) A1/A1	7.63 (6)	.267
	65 (29.3%) A1/A2	219 (31.9%) A1/A2	161 (38.4%) A1/A2		
	17 (7.7%) A2/A2	60 (8.7%) A2/A2	33 (7.9%) A2/A2		
	8 (3.6%) others	23 (3.4%) others	12 (2.9%) others		
APOE[b]	22 (9.7%) ε2/ε3	59 (8.6%) ε2/ε3	40 (9.6%) ε2/ε3	3.61 (6)	.730
	154 (68.1%) ε3/ε3	497 (72.4%) ε3/ε3	292 (70.0%) ε3/ε3		
	49 (21.7%) ε3/ε4	121 (17.6%) ε3/ε4	80 (19.2%) ε3/ε4		
	1 (0.4%) others	9 (1.3%) others	5 (1.2%) others		
ACE I/D	37 (16.3%) I/I	118 (17.2%) I/I	72 (17.1%) I/I	1.63 (4)	.804
	94 (41.4%) I/D	309 (45.0%) I/D	181 (43.1%) I/D		
	96 (42.3%) D/D	259 (37.8%) D/D	167 (39.8%) D/D		

APOE = apolipoprotein E; bp = base pairs; ACE = angiotensin coverting enzyme; D = deletion; df: degrees of freedom; HTR2A = serotonin receptor type 2A; IL-1α = interleukin-1 alpha; IL-1β = interleukin-1 beta; IL-1RA = interleukin-1 receptor antagonist; I = insertion; VNTR = variable number tandem repeat.

[a] Due to rounding, percentages may not sum exactly to 100%.

[b] 5-HTTLPR and IL-1α −889C/T genotypes unknown for one, IL-1β +3953C/T unknown for two, IL-1RA $(86\ bp)_n$ unknown for six, and APOE unknown for eight subjects.

Table 2
Genotype frequencies in suicide attempters by impulsivity and lethality.

		Impulsive attempt		Planned attempt		Low lethality		High lethality	
		n	(%)[a]	n	(%)[a]	n	(%)[a]	n	(%)[a]
HTR2A −1438A/G	A/A	25	(17.9%)	23	(31.5%)	29	(26.1%)	24	(22.2%)
	A/G	69	(49.3%)	39	(53.4%)	50	(45.0%)	58	(53.7%)
	G/G	46	(32.9%)	11	(15.1%)	32	(28.8%)	26	(24.1%)
χ^2 (df), p, corrected p		9.80 (2), 0.007, 0.056				1.64 (2), 0.440, >1.0			
5-HTTLPR[b]	L/L	39	(28.1%)	18	(24.7%)	34	(30.9%)	23	(21.3%)
	L/S	66	(47.5%)	38	(52.1%)	48	(43.6%)	60	(55.6%)
	S/S	34	(24.5%)	17	(23.3%)	28	(25.5%)	25	(23.1%)
χ^2 (df), p, corrected p		0.44 (2), 0.804, >1.0				3.61 (2), 0.165, >1.0			
STin2 VNTR	12/12	52	(37.1%)	33	(45.2%)	53	(47.7%)	36	(33.3%)
	12/10	66	(47.1%)	24	(32.9%)	35	(31.5%)	56	(51.9%)
	10/10	20	(14.3%)	15	(20.5%)	21	(18.9%)	15	(13.9%)
	Others	2	(1.4%)	1	(1.4%)	2	(1.8%)	1	(0.9%)
χ^2 (df), p, corrected p		4.24 (3), 0.237, >1.0				9.39 (3), 0.025, 0.200			
IL-1α −889C/T[b]	C/C	77	(55.0%)	39	(54.2%)	56	(50.9%)	63	(58.3%)
	C/T	56	(40.0%)	21	(29.2%)	44	(40.0%)	36	(33.3%)
	T/T	7	(5.0%)	12	(16.7%)	10	(9.1%)	9	(8.3%)
χ^2 (df), p, corrected p		8.76 (2), 0.013, 0.104				1.25 (2), 0.536, >1.0			
IL-1β +3953C/T[b]	C/C	82	(58.6%)	41	(57.7%)	61	(55.5%)	66	(61.7%)
	C/T	52	(37.1%)	24	(33.8%)	41	(37.3%)	35	(32.7%)
	T/T	6	(4.3%)	6	(8.5%)	8	(7.3%)	6	(5.6%)
χ^2 (df), p, corrected p		1.59 (2), 0.452, >1.0				0.92 (2), 0.633, >1.0			
IL-1RA (86 bp)$_n$[b]	A1/A1	76	(55.5%)	46	(64.8%)	62	(57.9%)	64	(59.8%)
	A1/A2	46	(33.6%)	17	(23.9%)	35	(32.7%)	28	(26.2%)
	A2/A2	9	(6.6%)	6	(8.5%)	6	(5.6%)	11	(10.3%)
	Others	6	(4.4%)	2	(2.8%)	4	(3.7%)	4	(3.7%)
χ^2 (df), p, corrected p		2.65 (3), 0.449, >1.0				2.28 (3), 0.516, >1.0			
APOE[b]	ε2/ε3	14	(10.1%)	7	(9.6%)	10	(9.1%)	12	(11.1%)
	ε3/ε3	93	(66.9%)	50	(68.5%)	76	(69.1%)	71	(65.7%)
	ε3/ε4	31	(22.3%)	16	(21.9%)	24	(21.8%)	24	(22.2%)
	ε4/ε4	1	(0.7%)	0	(0%)	0	(0%)	1	(0.9%)
χ^2 (df), p, corrected p		0.56 (3), 0.906, >1.0				1.33 (3), 0.721, >1.0			
ACE I/D	I/I	23	(16.4%)	11	(15.1%)	22	(19.8%)	13	(12.0%)
	I/D	57	(40.7%)	32	(43.8%)	47	(42.3%)	45	(41.7%)
	D/D	60	(42.9%)	30	(41.1%)	42	(37.8%)	50	(46.3%)
χ^2 (df), p, corrected p		0.20 (2), 0.904, >1.0				3.01 (2), 0.222, >1.0			

APOE = apolipoprotein E; bp = base pairs; ACE = angiotensin coverting enzyme; D = deletion; HTR2A = serotonin receptor type 2A; IL-1α = interleukin-1 alpha; IL-1β = interleukin-1 beta; IL-1RA = interleukin-1 receptor antagonist; I = insertion; VNTR = variable number tandem repeat.
Levels of impulsivity and lethality unknown for 14 and 8 suicide attempts, respectively.
[a] Due to rounding, percentages may not sum exactly to 100%.
[b] APOE, 5-HTTLPR and IL-1α −889C/T genotype unknown for one suicide attempter, IL-1β +3953C/T unknown for two, IL-1RA (86 bp)$_n$ unknown for five.

Table 3
Statistically significant associations between polymorphisms and impulsive and high lethality suicide attempts.

		Impulsive attempt		High lethality	
		OR	(95% CI)	OR	(95% CI)
Main effects					
HTR2A −1438A/G[a]	A/A	1.00	(Reference)		
	A/G	1.84	(0.90–3.74)		
	G/G	4.44**	(1.80–10.93)		
IL-1α −889C/T[a]	T/T	0.25**	(0.09–0.71)		
	C/T	1.38	(0.72–2.64)		
	C/C	1.00	(Reference)		
STin2 VNTR	12/12			1.00	(Reference)
	12/10			2.36**	(1.30–4.28)
	10/10			1.05	(0.48–2.31)
Interaction effects					
5-HTTLPR among suicide attempters aged 40 years+	L/L			1.00	(Reference)
	L/S			2.76	(0.91–8.39)
	S/S			7.78*	(1.56–38.76)
IL-1RA (86 bp)$_n$	A1/A1or A2/A2			1.00	(Reference)
with APOE ε3/ε3	A1/A2			0.32**	(0.14–0.69)
with APOE ε2/ε3 or ε3/ε4	A1/A2			2.98*	(1.01–8.79)

APOE = apolipoprotein E; bp = base pairs; ACE = angiotensin coverting enzyme; HTR2A = serotonin receptor type 2A; IL-1α = interleukin-1 alpha; IL-1RA = interleukin-1 receptor antagonist; VNTR = variable number tandem repeat.
[a] Associations estimated from logistic regression including both polymorphisms as independent variables.
* $p<0.05$.
** $p<0.01$.

3.2. Lethality of suicide attempt

Half (108, 49%) of the suicide attempts were of high lethality, overall and for men and women and young and old. Lethality of the suicide attempt was unrelated to psychiatric diagnosis [χ^2 (df) = 9.83 (7), p = 0.199].

STin2 VNTR genotype frequency trended toward being different in suicide attempts of low and high lethality [χ^2 (df) = 9.39 (3), uncorrected p = 0.025, corrected p = 0.200; Table 2]. Genotype 12/12 accounted for almost half (47.7%) of low lethality attempts but only one-third (33.3%) of high lethality attempts. Conversely, genotype 12/10 was associated with one-third (31.5%) of low lethality attempts and about half (51.9%) of high lethality attempts. STin2 VNTR was the only polymorphism to have a statistically significant main association with lethality of suicide attempt (Table 3). Compared to STin2 VNTR 12/12, the suicide attempt was more than twice as likely to be highly lethal with STin2 VNTR 12/10.

There was an interaction between the polymorphism 5-HTTLPR and age in relation to lethality of suicide attempt [LRT (df) = 6.84 (2), p = 0.033]. The frequency of 5-HTTLPR genotypes differed by lethality only among suicide attempters aged over 40 years [χ^2 (df) = 7.20 (2), p = 0.027]. The respective frequencies of the L/L, L/S, and S/S genotypes were 35.9%, 56.4%, and 7.7% for low lethality attempts and 14.3%, 61.9%, and 23.8% for high lethality attempts. Thus, among older suicide attempters, the S allele was associated with high lethality [54.8% vs. 35.9%; χ^2 (df) = 5.80 (1), p = 0.016; OR = 2.16, 95% CI = 1.15–4.08]. In terms of genotype, there was a graded association with lethality (Table 3). Compared to the reference genotype L/L, the risk of a highly lethal suicide attempt was higher for the L/S genotype and far higher for the S/S genotype.

There was also evidence of interaction between the polymorphisms APOE and IL-1RA (86 bp)$_n$ [LRT (df) = 12.21 (4), p = 0.016]. There was evidence of an association between IL-1RA (86 bp)$_n$ and lethality both in the presence of the APOE ε3/ε3 genotype [χ^2 (df) = 12.21 (4), p = 0.016] and when only one of the APOE genotype alleles was ε3 [i.e. ε2ε3 or ε3ε4; χ^2 (df) = 12.21 (4), p = 0.016]. With the former, IL-1RA (86 bp)$_n$ A1/A2 was associated with reduced risk of highly lethal attempted suicide whereas with the latter, it was associated with increased risk of high lethality (Table 3).

3.3. Haplotype analysis

The independent haplotype analysis of SLC6A4 and IL-1 gene polymorphisms revealed no significant differences between suicide attempters and controls. However, the IL-1T/C/A2 haplotype was more frequent in the control group than in the suicide attempters [3.4% vs. 1.4%, χ^2 (df) = 5.14 (1), p = 0.023; OR = 0.40, 95% CI = 0.18–0.91], mainly due to haplotype differences between the psychiatric control group and the suicide attempters [3.9% vs. 1.4%, χ^2 (df) = 6.39 (1), p = 0.011; OR = 0.35, 95% CI = 0.15–0.82]. No haplotype differences were found related to suicide attempt impulsivity or lethality.

4. Discussion

In this study, no association was found between the studied polymorphisms and suicidal behavior. We, however, found a trend towards an excess of the −1438G/G (and −1438G allele) and a deficit of IL-1α −889T/T genotypes in impulsive attempts. We also found an interaction between the 5-HTTLPR and age in relation to lethality (among older suicide attempters, the S allele was associated with high lethality in a dose–effect way), and between the polymorphisms APOE and IL-1RA (86 bp)$_n$ (higher lethality in the presence of IL-1RA heterozygosity and APOE ε2ε3 or ε3ε4 genotypes). However, no gene by gene interactions between functional polymorphisms of genes directly related to serotonergic neurotransmission (i.e., HTR2A −1438A/G and SLC6A4 5-HTTLPR and STin2 VNTR) were found.

Related to the HTR2A polymorphism our results confirm those of case–control association (Khait et al., 2005) and meta-analytic studies (Anguelova et al., 2003). The excess of −1438G allele in impulsive attempts are in line with the findings that the rs6311 C allele is overrepresented in patients with impulsive suicide attempts (Giegling et al., 2006; Zalsman et al., 2010). Overall, these data suggest that this functional polymorphism may modify the phenotype of suicidal behavior and could be related to the impulsivity of the attempt.

Our results agree with data confirming no association between suicide attempt and the SLC6A4 polymorphisms (Baca-Garcia et al., 2004a; Courtet et al., 2003; Rujescu et al., 2001). Lack of concordance with meta-analytic evidences (Anguelova et al., 2003; Li and He, 2007; Lin and Tsai, 2004) concluding that the S allele is involved in susceptibility to suicidal behavior might be explained by the small number of violent attempters in our sample. However, our data agree, at least in part, with other case–control association reports suggesting a possible effect of the S allele on the severity of suicide attempts (Courtet et al., 2004; Gorwood et al., 2000; Wasserman et al., 2007). Altogether, these findings are in line with the fact that, in suicide attempters, low 5-HTT availability is associated with the S allele (Bah et al., 2008). Data regarding the functional role of the STin2 VNTR have led some studies to suggest that the STin2.12 allele acts as a transcriptional enhancer (MacKenzie and Quinn, 1999) and others to suggest that this allele is associated with low 5-HTT availability (Bah et al., 2008).

To our knowledge, the only study analyzing the association between functional polymorphisms in IL-1 gene complex and suicidal behavior was conducted by our group using a sample of fewer suicide attempters (n = 193) (Saiz et al., 2008b) that mostly overlaps the current sample. The previous and present studies found no association between suicidal behavior and the IL-1 gene when analyzing the polymorphisms one by one, in line with other reports showing no differences in serum IL-1β between depressed patients with and without suicidal behavior (Huang and Lee, 2007) or no changes in the expression of IL-1β in the orbitofrontal cortex in suicide victims (Tonelli et al., 2008). The regulatory effect of the IL-1α −889C/T on basal IL-1β production (Hulkkonen et al., 2000) and a possible overexpression of IL-α associated with the −889T allele (Du et al., 2000), might explain the trend towards a deficit of the −889T/T genotype found in impulsive suicide attempts.

Our results are in line with the findings that genes encoding proteins involved in cholesterol biosynthesis and transport are not associated with suicide in Caucasians (Lalovic et al., 2004), and that plasma APOE levels may not be associated with suicide attempts despite low cholesterol being associated with suicide attempts (Baca-Garcia et al., 2004b). However, the finding of an enhanced IL-1β production in vitro associated with the presence of the IL-1RA A2 allele (Santtila et al., 1998) as well as the role of the IL-1β in the stimulation of neuronal 5-HTT activity (Zhu et al., 2006), might be related to the reported interaction between the IL-1RA (86 bp)$_n$ and the APOE genotypes on the lethality of the attempt. Taking into account the prior suggested associations between the APOE genotypes and serum cholesterol levels (Liu et al., 1999), the real mechanism underlying this interaction is as yet unknown.

Finally, our data suggest no role for the ACE I/D polymorphism in suicidal behavior and agree with Hong et al. (2002), who found no differences in genotype frequencies between major depressive patients with and without a history of suicide attempts. Nevertheless, further replications are needed, as positive discrepant findings have been reported (Hishimoto et al., 2006; Sparks et al., 2009).

The study had some limitations. Firstly, it is possible that due to small sample size our study had insufficient statistical power to detect genuinely significant associations with a small effect size (i.e., Type II error). On the other hand, there was a risk of false positive results due to population stratification because of deviation from Hardy–Weinberg equilibrium in suicide attempters [IL-1RA (86 bp)$_n$] and

psychiatric controls [STin2 VNTR and IL-1RA $(86\ bp)_n$]. In addition, it is possible that there were some bias or genotyping errors. However, our patients were of Caucasian Spanish origin and were comparable with respect to the geographic origin of their families. Furthermore, in order to avoid genotyping errors, the different groups of patients were analyzed in parallel and blind to clinical condition and randomized subjects were re-tested for their genotypes confirming reproducibility. It is also possible that the inclusion of other candidate genes related to the serotonergic neurotransmission could help better understand the genetic basis of suicidal behavior. Another possible limitation is that different psychiatric diagnoses were included in the suicide attempt sample. However, we included a non-suicide attempt psychiatric control group. Furthermore, we agree with earlier reports that suggest that vulnerability to suicidal behavior may be familially transmitted as a trait independent of Axis I and II disorders (Brent and Mann, 2005). Finally, related to the 5-HTTLPR polymorphism, due to limited availability of DNA for genotyping, we were unable to examine the A→G substitution within the L allele in our samples.

5. Conclusion

We found no differences in the studied polymorphisms between suicide attempters and psychiatric and healthy controls. However, we found at least partial evidence that the impulsivity and lethality of attempted suicide may be influenced by specific polymorphisms as well as by gene–gene relationships and interactions between genes and non-genetic variables. These findings are further evidence of the complexity of the association between genetics and suicidal behavior. They support the approach of studying more homogenous forms of suicidal behavior such as impulsive or medically serious attempts and the more recent suggested approach of seeking to identify endophenotypes for suicidal behavior of which impulsive and aggressive traits appear particularly promising (Mann et al., 2009).

Acknowledgments

This study was supported in part by Oviedo University grants NP-01-519-3 and MB-05-519, and the Instituto de Salud Carlos III, Centro de Investigacion Biomedica en Red de Salud Mental, CIBERSAM.

References

Anguelova M, Benkelfat C, Turecki G. A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: II. Suicidal behavior. Mol Psychiatry 2003;8:646–53.
Asberg M, Träskman L, Thorén P. 5-HIAA in the cerebrospinal fluid. A biochemical suicide predictor? Arch Gen Psychiatry 1976;33:1193–7.
Asensi V, Alvarez V, Valle E, Meana A, Fierer J, Coto E, et al. IL-1 alpha (−889) promoter polymorphism is a risk factor for osteomyelitis. Am J Med Genet A 2003;119A: 132–6.
Baca-Garcia E, Vaquero C, Diaz-Sastre C, Garcia-Resa E, Saiz-Ruiz J, Fernandez-Piqueras J, et al. Lack of association between the serotonin transporter promoter gene polymorphism and impulsivity or aggressive behavior among suicide attempters and healthy volunteers. Psychiatry Res 2004a;126:99-106.
Baca-Garcia E, Diaz-Sastre C, Garcia-Resa E, Ceverino A, Ramirez A, Saiz-Ruiz J, et al. Lack of association between plasma apolipoprotein E and suicide attempts. J Clin Psychiatry 2004b;65:580.
Baca-Garcia E, Rodriguez Salgado B, Dolengevich Segal H, Vaquero Lorenzo C, Navio Acosta M, Arrojo Romero M, et al. A pilot genetic study of the continuum between compulsivity and impulsivity females: the serotonin transporter promoter polymorphism. Prog Neuropsychopharmacol Biol Psychiatry 2005;29:713–7.
Bah J, Lindström M, Westberg L, Mannerås L, Ryding E, Henningsson S, et al. Serotonin transporter gene polymorphisms: effect on serotonin transporter availability in the brain of suicide attempters. Psychiatry Res 2008;162:221–9.
Beck AT, Schuyler D, Herman I. Development of suicidal intent scales. In: Beck AT, Resnick HLP, Lettieri DJ, editors. The prediction of suicide. Bowie: Charles Press; 1974. p. 45–56.
Beck AT, Beck R, Kovacs M. Classification of suicidal behaviors. I. Quantifying intent and medical lethality. Am J Psychiatry 1975;132:285–7.
Bellivier F, Szoke A, Henry C, Lacoste J, Bottos C, Nosten-Bertrand M, et al. Possible association between serotonin transporter gene polymorphism and violent suicidal behavior in mood disorders. Biol Psychiatry 2000;48:319–22.
Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995;310:170.
Bondy B, Erfurth A, de Jonge S, Kruger M, Meyer H. Possible association of the short allele of the serotonin transporter promoter gene polymorphism (5-HTTLPR) with violent suicide. Mol Psychiatry 2000;5:193–5.
Bondy B, Buettner A, Zill P. Genetics of suicide. Mol Psychiatry 2006;11:336–51.
Brent DA, Mann JJ. Family genetic studies, suicide, and suicidal behavior. Am J Med Genet C Semin Med Genet 2005;133:13–24.
Courtet P, Baud P, Abbar M, Boulenger JP, Castelnau D, Mouthon D, et al. Association between violent suicidal behavior and the low activity allele of the serotonin transporter gene. Mol Psychiatry 2001;6:338–41.
Courtet P, Buresi C, Abbar M, Baud P, Boulenger JP, Castelnau D, et al. No association between non-violent suicidal behavior and the serotonin transporter promoter polymorphism. Am J Med Genet B Neuropsychiatr Genet 2003;116:72–6.
Courtet P, Picot MC, Bellivier F, Torres S, Jollant F, Michelon C, et al. Serotonin transporter gene may be involved in short-term risk of subsequent suicide attempts. Biol Psychiatry 2004;55:46–51.
Cox A, Camp NJ, Nicklin MJ, di Giovine FS, Duff GW. An analysis of linkage disequilibrium in the interleukin-1 gene cluster, using a novel grouping method for multiallelic markers. Am J Hum Genet 1998;62:1180–8.
Diaz FJ, Baca-Garcia E, Diaz-Sastre C, Garcia Resa E, Blasco H, Braquehais Conesa D, et al. Dimensions of suicidal behavior according to patient reports. Eur Arch Psychiatry Clin Neurosci 2003;253:197–202.
Du Y, Dodel RC, Eastwood BJ, Bales KR, Gao F, Lohmuller F, et al. Association of an interleukin 1 alpha polymorphism with Alzheimer's disease. Neurology 2000;55: 464–5.
Dunn AJ, Swiergiel AH, de Beaurepaire R. Cytokines as mediators of depression: what can we learn from animal studies? Neurosci Biobehav Rev 2005;29:891–909.
Giegling I, Hartmann AM, Möller HJ, Rujescu D. Anger- and aggression-related traits are associated with polymorphisms in the 5-HT-2A gene. J Affect Disord 2006;96: 75–81.
Gorwood P, Batel P, Ades J, Hamon M, Boni C. Serotonin transporter gene polymorphisms, alcoholism, and suicidal behavior. Biol Psychiatry 2000;48:259–64.
Hishimoto A, Shirakawa O, Nishiguchi N, Hashimoto T, Yanagi M, Nushida H, et al. Association between a functional polymorphism in the renin–angiotensin system and completed suicide. J Neural Transm 2006;113:1915–20.
Hong CJ, Wang YC, Tsai SJ. Association study of angiotensin I-converting enzyme polymorphism and symptomatology and antidepressant response in major depressive disorders. J Neural Transm 2002;109:1209–14.
Huang TL, Lee CT. T-helper 1/T-helper 2 cytokine imbalance and clinical phenotypes of acute-phase major depression. Psychiatry Clin Neurosci 2007;61:415–20.
Hulkkonen J, Laippala P, Hurme M. A rare allele combination of the interleukin-1 gene complex is associated with high interleukin-1 beta plasma levels in healthy individuals. Eur Cytokine Netw 2000;11:251–5.
Jenkins TA, Allen AM, Chai SY, Mendelsohn FA. Interactions of angiotensin II with central catecholamines. Clin Exp Hypertens 1995;17:267–80.
Khait VD, Huang YY, Zalsman G, Oquendo MA, Brent DA, Harkavy-Friedman JM, et al. Association of serotonin 5-HT2A receptor binding and the T102C polymorphism in depressed and healthy Caucasian subjects. Neuropsychopharmacology 2005;30: 166–72.
Lalovic A, Sequeira A, DeGuzman R, Chawky N, Lesage A, Seguin M, et al. Investigation of completed suicide and genes involved in cholesterol metabolism. J Affect Disord 2004;79:25–32.
Li D, He L. Meta-analysis supports association between serotonin transporter (5-HTT) and suicidal behaviour. Mol Psychiatry 2007;12:47–54.
Lin PY, Tsai G. Association between serotonin transporter gene promoter polymorphism and suicide: results of a meta-analysis. Biol Psychiatry 2004;55:1023–30.
Liu HC, Hong CJ, Wang SJ, Fuh JL, Wang PN, Shyu HY, et al. ApoE genotype in relation to AD and cholesterol: a study of 2326 Chinese adults. Neurology 1999;53:962–6.
MacKenzie A, Quinn J. A serotonin transporter gene intron 2 polymorphic region, correlated with affective disorders, has allele-dependent differential enhancer-like properties in the mouse embryo. Proc Natl Acad Sci USA 1999;96:15251–5.
Mann JJ, Waternaux C, Haas GL, Malone KM. Toward a clinical model of suicidal behavior in psychiatric patients. Am J Psychiatry 1999;156:181–9.
Mann JJ, Arango VA, Avenevoli S, Brent DA, Champagne FA, Clayton P, et al. Candidate endophenotypes for genetic studies of suicidal behavior. Biol Psychiatry 2009;12: 327–35.
Martínez-Barrondo S, Saiz PA, Morales B, García-Portilla MP, Coto E, Alvarez V, et al. Serotonin gene polymorphisms in patients with panic disorder. Actas Esp Psiquiatr 2005;33:210–5.
Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
Nomura M, Kusumi I, Kaneko M, Masui T, Daiguji M, Ueno T, et al. Involvement of a polymorphism in the 5-HT2A receptor gene in impulsive behavior. Psychopharmacology (Berl) 2006;187:30–5.
Poirier J. Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. Trends Neurosci 1994;17:525–30.
Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res 1992;20:1433.
Rujescu D, Giegling I, Sato T, Moeller HJ. A polymorphism in the promoter of the serotonin transporter gene is not associated with suicidal behavior. Psychiatr Genet 2001;11:169–72.
Rujescu D, Thalmeier A, Möller HJ, Bronisch T, Giegling I. Molecular genetic findings in suicidal behavior: what is beyond the serotonergic system? Arch Suicide Res 2007;11:17–40.

Saiz PA, Bobes J, Gonzalez MP, Cocaña I, Gonzalez-Quiros P, Bousoño M. Searching for a predictive peripherical biological model in parasuicidal behaviour. Eur Psychiatry 1997;12:75–81.

Saiz PA, Garcia-Portilla MP, Paredes B, Arango C, Morales B, Alvarez V, et al. Association between the A-1438G polymorphism of the serotonin 2A receptor gene and nonimpulsive suicide attempts. Psychiatr Genet 2008a;18:213–8.

Saiz PA, Garcia-Portilla P, Paredes B, Arango C, Morales B, Alvarez V, et al. Association study of the interleukin-1 gene complex and tumor necrosis factor alpha gene with suicide attempts. Psychiatr Genet 2008b;18:147–50.

Santtila S, Savinainen K, Humer M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1β production in vitro. Scand J Immunol 1998;47:195–8.

Sarchiapone M, Roy A, Camardese G, De Risio S. Further evidence for low serum cholesterol and suicidal behaviour. J Affect Disord 2000;61:69–71.

Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005;15:97–8.

Sparks DL, Hunsaker 3rd JC, Amouyel P, Malafosse A, Bellivier F, Leboyer M, et al. Angiotensin I-converting enzyme I/D polymorphism and suicidal behaviors. Am J Med Genet B Neuropsychiatr Genet 2009;150B:290–4.

Tarlow JK, Blakemore AI, Lennard A, Solari R, Hughes HN, Steinkasserer A, et al. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet 1993;91:403–4.

Tiret L, Rigat B, Visvikis S, Breda C, Corvol P, Cambien F, et al. Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. Am J Hum Genet 1992;51: 197–205.

Tonelli LH, Stiller J, Rujescu D, Giegling I, Schneider B, Maurer K, et al. Elevated cytokine expression in the orbitofrontal cortex of victims of suicide. Acta Psychiatr Scand 2008;117:198–206.

Wasserman D, Geijer T, Sokolowski M, Frisch A, Michaelovsky E, Weizman A, et al. Association of the serotonin transporter promotor polymorphism with suicide attempters with a high medical damage. Eur Neuropsychopharmacol 2007;17: 230–3.

Wenham PR, Price WH, Blandell G. Apolipoprotein E genotyping by one-stage PCR. Lancet 1991;337:1158–9.

World Health Organisation. Summary report, working group in preventive practices in suicide and attempted suicide. Copenhagen: WHO Regional Office for Europe; 1986.

Xhignesse M, Lussier-Cacan S, Sing CF, Kessling AM, Davignon J. Influences of common variants of apolipoprotein E on measures of lipid metabolism in a sample selected for health. Arterioscler Thromb 1991;11:1100–10.

Zalsman G, Patya M, Frisch A, Ofek H, Schapir L, Blue I, et al. Association of polymorphisms of the serotonergic pathways with clinical traits of impulsive-aggression and suicidality in adolescents: a multi-center study. World J Biol Psychiatry 2010 [Epub ahead of print].

Zhu CB, Blakely RD, Hewlett WA. The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. Neuropsychopharmacology 2006;31:2121–31.