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ORIGINAL PAPER

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A pilot study on a gene-hormone interaction in female suicide attempts

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Abstract This one-year naturalistic study included all suicide attempters in a catchment area. In the first published set of analyses, an association between menses and suicide attempts was replicated. According to the polymorphism of the serotonin transporter promoter area, the subjects can be classified as S individuals (s/s or s/l) or L individuals (l/l). In the second published set of analyses, L females appeared protected from suicide attempts since they were underrepresented among female (and not male) attempters. This new, unpublished third set of analyses tested for an interaction between the same polymorphism and low hormonal activity (during menses and menopause). In fertile female attempters, the proportion of L women in the menses (41 %, 7/17) was significantly higher than expected in the population (15.5 %) and almost significantly higher than in S female attempters (22 %, 19/87). L females were also overrepresented in postmenopausal attempters. Despite sample size limitations, this gene-hormone interaction needs to be further investigated in female suicide attempters.

Key words genetics · serotonin · menstrual cycle · menstruation · estrogens · menopause

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Introduction

Suicide attempts are more frequent in women than in men, but men are more likely to complete suicide. Suicide attempts, in more than 90 % of the cases, are associated with psychiatric disorders. Vulnerability to suicide is probably best understood in the context of a model of stress-diathesis for suicidal behavior (Mann 1998). The diathesis for suicide behavior is influenced by genetics, rearing, chronic illness, chronic substance abuse and possibly cholesterol level. Typical stressors include psychiatric illness, acute use of alcohol or sedatives, acute medical illness, and acute family and social stress.

Growing evidence indicates that genetic factors contribute to suicide risk. Reviews of the literature suggest a heritability of suicide of about 43–45 % (Statham et al. 1998). Familial and adoption studies suggest that suicide behavior appears to be transmitted as a trait independent of Axis I and II disorders. Statham et al. (1998) suggested that genetic factors can be detected after correcting for psychiatric diagnosis, traumatic events, personality traits, and religious affiliations.

Low brain serotonergic function appears to be undeniably related to suicidal behavior across different psychiatric diagnoses and within specific psychiatric diagnoses (Mann 1998). Thus, polymorphic variations in the serotonergic system are ideal gene candidates for genetic studies in suicide. The serotonin transporter (5-HTT) gene is one of the major genes known to influence serotonergic transmission. It was mapped to the chromosome 17q11.1-q12. In the promoter area, there are two allelic variants, a long (l) variant and a short (s) variant. In vitro studies show that the "l" allele has 2 to 3

times higher basal transcriptional activity. The "s" allele acts in a nearly dominant way. Therefore, the subjects are classified as S individuals (s/s or s/l) or L individuals (l/l) (Heils et al. 1996). S individuals have lower serotonergic activity according to clinical (Heinz et al. 2000; Reist et al. 2001; Williams et al. 2001) and animal studies (Lesch and Heils 2000).

Many studies relating the promoter area 5-HTT genotype and psychiatric illnesses have been negative; a few were positive but have not been replicated. There are nine published studies relating the 5-HTT genotype with suicidal behavior on specific psychiatric diagnoses. Four studies have been negative: one on completed suicide (Fitch et al. 2001), one on completed depressive suicides (Mann et al. 2000), one on attempted suicide (Geijer et al. 2000), and one on subjects believed to have high suicidal risk (Russ et al. 2000). One very small study on completed suicide in depression showed an increase of L subjects (Du et al. 1999). Five studies showed an increase of S subjects in attempters: one study in completed suicides (Bondy et al. 2000), one study on violent suicide attempts in depressive patients (Bellivier et al. 2000), one study on more lethal attempters in alcoholic patients (Gorwood et al. 2000), one study on suicide attempts in alcoholics (Preuss et al. 2001) and the other on violent suicide attempts, most of them in depressive patients (Courtet et al. 2001). None of these studies explored gender differences.

The menstrual cycle may influence suicidal behavior. Our extensive review of the literature included 17 prior studies published during the last 40 years, and proposed that most of the studies suggested that the time around the menses is a critical period for suicidal behavior (Baca-Garcia et al. 2000). Most authors thought that the menstrual cycle could be considered as another contributing factor for suicide. Forrestie et al. (1986) hypothesized that the low estrogen levels during menses may contribute to suicide attempt. Therefore, in Mann's suicide model, the menses will act as a stressor. In effect, our prior study from a catchment area of a general hospital verified a significant association between the menses and suicide attempts (Baca-Garcia et al. 2000).

This study, in the same hospital, was designed to test the possibility of a menses interaction with the promoter area polymorphism of the serotonin transporter in female suicide attempts. The third set of analyses described in this article was conducted after completing two prior analyses sets establishing: 1) the replication of the association between the menses and suicide attempts (Baca-Garcia et al. 2003); and 2) an association between the promoter area polymorphism of the serotonin transporter and female suicide attempts (Baca-Garcia et al. 2002).

In the first published analysis set, we found a significant increase of 1.72 in probability of attempted suicide during the menses when compared with the duration of the menses in controls (Baca-Garcia et al. 2003). In the second published analyses set, we found a dimorphic association with suicide attempts (Baca-Garcia et al. 2002). In males, the polymorphism in the promoter area of the serotonin transporter was not associated with suicide attempts. However, in female attempters, L individuals were signficantly underrepresented (21 % vs 35 % in the female controls) (Baca-Garcia et al. 2002).

This unpublished third set of analyses tests the possibility of an interaction on suicide between the menses and the polymorphism in the promoter area of the serotonin transporter in a smaller sample including fertile females. As described previously, it is hypothesized that low estrogen levels during the menses may contribute to the association between the menses and suicide attempts (Forestie et al. 1986). Low estrogen levels during menses may be associated with a further decrease in brain serotonergic activity, contributing to increased suicide risk in females vulnerable to suicide attempts. A genetic variability may make some females more vulnerable to the drop in estrogen levels. A recent study suggested that L individuals might be more sensitive to tryptophan depletion (Moreno et al. 2002). In our model, L women will also be more sensitive to the effects of low estrogen levels; therefore, they will be especially vulnerable to suicide during the menses. Menopause is another major time period of low hormone levels in the female life cycle. Thus, if there is an interaction in suicide between the menses and the polymorphism in the promoter area of the serotonin transporter in fertile females, the same interaction should happen in menopausal attempters. Therefore, in our model, L women will also be more sensitive to the effects of low estrogen levels and especially vulnerable to suicide during menopause. In summary, the idea of the interaction in our model can be summarized: S females are more vulnerable to suicide attempts in general; however, L females are more vulnerable to suicide attempts when hormone levels are low (menses and menopause).

Methods

Subjects

Between February 1999 and January 2000 (Baca-Garcia et al. 2002, 2003), all consenting suicide attempters were prospectively recruited at the Hospital Ramon y Cajal. This general hospital of the National Health Service provides free medical coverage to the population and triages all emergencies in a catchment area of 500,000 persons in Madrid (Spain). All subjects were Caucasians. Suicide attempters were assessed during the first 24 hours after the attempt and were defined, as the US National Institute of Health recommends, as those attempts with some evidence that the person intended to kill himself/herself (O'Carroll et al. 1996). The Mini International Neuropsychiatric Interview (MINI) version 4.4 was used to establish Axis I psychiatric disorders in DSM-IV. After completely describing the study to the subjects, written informed consent was obtained.

Assessment of the menstrual cycle

The hospital laboratory analyzed samples collected in the emergency room during the first 24 hours for sexual hormones (estradiol, progesterone, LH and FSH) using chemoluminescent enzyme immunoassay. The results have been described previously (Baca-Garcia et al. 2003).

All fertile attempters were asked about the average duration of menstruation and of the menstrual cycle for 3 prior cycles. Subjects with unusual hormone levels, irregular menstrual cycles or use of hormonal contraceptives were excluded (Baca-Garcia et al. 2003). There were 104 female attempters with regular menstrual cycles, not currently on hormonal contraceptives who had completed genotypes and were included in the analyses of this article. The attempters were then classified according to their report, verified by hormone levels, as being in the menses or the non-menstrual phases of the menstrual cycle. An additional 21 genotyped menopausal attempters were used for a replication analysis of the effect of low hormone levels. The clinical characteristics of the samples are described in Table 1.

A ratio of the menses duration divided by menstrual cycle duration was calculated in each individual control (160 blood donors at the same hospital) to establish the probability of the general population's menses. Each individual's ratio was calculated by dividing the average duration of the last 3 menses by the average duration of the last 3 menstrual cycles. The control group mean was obtained by averaging individual's ratios. This mean ratio, 0.155 or 15.5 %, was used as the expected probability that a woman of the general population is in the menses (Baca-Garcia et al. 2003).

Table 1 Sociodemographic and clinical data of the suicide attempters

Variables	Fertile during menses (N = 26)	Fertile not during menses (N = 78)	Menopausal (N = 21)
Age	30.2 year	30.7 year	63.4 year
Marital status			
single	52%	47%	6%
married	32%	33%	77%
divorced	16%	20%	17%
Had children	36%	40%	94%
Educational level			
primary	28%	39%	89%
high school	56%	34%	6%
college	16%	27%	5%
Occupation			
housewife	13%	12%	78%
student	29%	12%	0%
employed	58%	72%	22%
self-employed	0%	4%	0%
Working status:			
unemployed	32%	35%	17%
disabled	16%	19%	44%
working	52%	46%	39%
AXIS-I DIAGNOSIS			
Any axis-I diagnosis	92%	95%	83%
Most frequent axis-I diagnosis (> 10 %)			
Major depressive episode	64%	62%	30%
Dysthymia	4%	5%	24%
Generalized anxiety disorder	16%	8%	12%
Alcohol dependence or abuse	16%	26%	0%
Drug dependence or abuse	24%	5%	6%
Eating disorders	16%	24%	0%
Adjustment disorder	12%	8%	18%
Prior suicide attempts			
none	42%	38%	50%
0–2	42%	28%	40%
> 2	16%	24%	10%
Method			
self-poisoning	74%	93%	84%

Genotype determinations

As described in the second set of analyses in attempters and controls (Baca-Garcia et al. 2002), DNA was extracted from a 10 ml blood sample according to standard methods. Subjects were screened by the molecular analysis of a particular insertion/deletion polymorphism in the promoter region of the 5-HTT gene. Target DNA was amplified using the primers and conditions previously described (Heils et al. 1996). Amplified products were resolved in agarose gels and detected by ethidium bromide staining. Subjects were classified as S and L.

Results

In 17 L fertile female attempters, the proportion of women in the menses, 41 % (7/17), was significantly higher (Exact, 1-tailed p = 0.02) when compared with 15.5 % expected from the population. In 87 S fertile female attempters, the proportion of women in the menses, 22 % (19/87), exceeded the expected 15.5 %, but did not reach significance (z = 1.5, 1-tailed p = 0.15). When the proportion of attempters in the menses was compared in L attempters, 41 %, versus S attempters, 22 %, the difference almost reached significance ($\chi^2 = 2.8$, df = 1, p = 0.09).

To verify that low hormone levels may influence L females more than S females, postmenopausal attempters were compared with fertile attempters (Table 2). As expected, the L genotype frequency was higher in menopausal attempters (38 %) than in fertile attempters (16 %) ($\chi^2 = 5.3$, df = 1, p = 0.002). The L genotype frequency (38 %) was higher in menopausal attempters than in fertile attempters during the non-menstrual phases of the menstrual cycle with higher hormone levels (13 %) ($\chi^2 = 7.7$, df = 1, p = 0.005) (Table 2). However, it was not significantly different from the L genotype frequency in fertile attempters during the menses (27 %) ($\chi^2 = 2.4$, df = 1, p = 0.12).

Table 2 Distribution of the serotonin transporter promoter phenotypes in fertile and postemenopausal attempters

	L females N (%) *l/l*	S females N (%)		TOTAL N (%)
		s/s	*s/l*	
FERTILE				
In menses	7 (27%)	19 (73%)		26 (100%)
		5 (19%)	*14 (54%)*	
Other phases	10 (13%)	68 (87%)		78 (100%)
		33 (42%)	*35 (45%)*	
Total	17 (16%)	87 (84%)		104 (100%)
		38 (37%)	*49 (47%)*	
MENOPAUSAL	8 (38%)	13 (62%)		21 (100%)
		3 (14%)	*10 (48%)*	

Estradiol and progesterone levels during the attempt were compared in all attempters to explore whether low hormone levels may influence L females vulnerable to suicide attempts more than S females vulnerable to suicide attempts (Table 3). Estradiol levels were significantly lower in the L attempters (Mann-Whitney $U = 880$; $Z = 2.2$; $p = 0.03$).

Discussion

This study has all the limitations of prospective naturalistic studies without an experimental design. The strength of including all consenting suicide attempters from a catchment area has been described before (Baca-Garcia et al. 2003). The limitations of using a single hormonal determination, and those of any study on the relationship between the menstrual cycle and suicide, are discussed in detail in prior articles (Baca-Garcia et al. 2000, 2003).

The main specific limitation of this third set of analyses is that they are limited by the borderline significance in some of the analyses and the limited sample size. However, it must be remembered that recruiting enough L females attempters is inherently difficult since they are underrepresented among L fertile female attempters. The original sample included almost 200 attempts. After excluding males and non-fertile females, only 17 L fertile females attempters were recruited due to the intense underrepresentation of L attempters among fertile female attempters. It was remarkable that the results on postmenopausal females similarly suggested that low hormone levels may be more detrimental for L females with vulnerability to suicide attempts. The small number of L attempters (in fertile and postmenopausal females) precludes the possibility of conducting further analyses by controlling for other clinical variables.

In order to replicate the study in the same hospital, we currently estimate that approximately four years of recruitment are needed for enough statistical power to test the two hypotheses that did not reach significance (Borenstein et al. 2001). After four years of recruitment, the sample size (67 L fertile attempters and 348 S fertile attempters) would have a power of 87 % to establish significant differences between the 41 % of L females attempting suicide during the menses versus the 22 % of S females. This sample size would also have a power of 88 % to find significant differences between the 22 % of S females attempting suicide during the menses versus the expected 15.5 % from the population.

Gender differences in serotonergic function have been described. Animal studies have provided conflicting results, but generally suggest an increase of serotonergic activity in the female rat brain. Human studies suggested that females may have higher serotonergic activity (Mann 1998). Finally, anatomical sexual dimorphism has been suggested (Cordero et al. 2000) in the raphe nuclei, the area with higher density of serotonin transporter (Frazer et al. 1999).

Many years ago, Forestie et al. (1986) specifically hypothesized that low estrogen levels may contribute to suicide. The review of basic and clinical studies has led us to hypothesize that Forestie et al. may be correct in that low estrogen levels during menses in vulnerable females may be associated with a further decrease in brain serotonergic activity contributing to increased suicide risk (Baca-Garcia et al. 2003). Moreno et al. (2002) described that L individuals are more sensitive to tryptophan depletion. Similarly, this article suggests that, although L females may be protected from suicide in general, they are more vulnerable to suicide during decreases of estradiol during the menses or menopause.

Replication of our results on the interaction between L genotype and hormone levels would suggest that L females may be protected from suicide attempts when compared with S females. However, in situations of low hormone levels (menstrual phase or menopause), L females may lose this protection. Although this study certainly needs replication in a similar representative sample of female attempters, it suggests that gene-hormone interaction may be important in understanding female suicide attempts. It may be difficult to develop a study to replicate our findings; our catchment area includes a genetically homogenous population, a very low number of women taking hormonal contraceptives and a high percentage of suicide attempters willing to sign the consent form.

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Table 3 Comparison of estradiol and progesterone levels in suicide attempters using Mann-Whitney test

		Estradiol (pg/ml)				Progesterone (ng/mL)			
	N	Median	mean	SD	p value	Median	mean	SD	p value
L	25	46.8	63.8	82.2	0.03	0.9	1.7	2.5	0.21
S	100	66.1	82.1	64.8		1.2	2.9	14.8	

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