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Epigenetics-by-sex interaction for somatization conferred by methylation at the promoter region of *SLC6A4* gene

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ABSTRACT

Background: Depression, anxiety and somatoform disorders are all more prevalent in women than in men. However, specific biological mechanisms contributing to such sex differences remain unknown. Serotonergic pathways are involved in mood and behavior regulation and thus have been suggested to be altered in several psychiatric disorders. The serotonin transporter (SERT), encoded by *SLC6A4* gene, has received major attention due to its crucial role in serotonergic transmission.
Methods: 148 monozygotic twin subjects were assessed for (i) lifetime categorical diagnosis of anxious-depressive disorders, following SCID-I-based DSM-IV criteria, and (ii) current psychiatric symptomatology, from a dimensional approach, by means of the Brief Symptom Inventory (BSI). *SLC6A4* gene methylation was analyzed by means of Infinium HumanMethylation450 in a subset of the sample. CpG-specific methylation at the promoter region of *SLC6A4* gene was further analyzed by means of pyrosequencing technology in the total sample.
Results: *SLC6A4* methylation was found to be significantly higher in women when compared to men independent of DSM-IV diagnosis. *SLC6A4* methylation was further associated with the BSI-derived somatization dimension.
Conclusions: Female hypermethylation of a discrete region located within *SLC6A4* promoter region could underlie differential SERT expression in women when compared to men and could be one of the causative mechanisms by which women exhibit increased prevalence of somatic symptoms.

1. Introduction

There are well-established sex differences in the prevalence of certain mental disorders. Internalizing disorders are more common among women and externalizing disorders among men (Boyd et al., 2015). Within internalizing disorders, the burden of mood, anxiety and somatoform disorders is far greater among women than men (Kessler et al., 1994). What accounts for sex disparities in the prevalence of these disorders is currently unknown (Kuehner, 2017; van Loo et al., 2017). In this regard, epigenetic mechanisms and, particularly, DNA methylation pattern have been described to be greatly influenced by stochastic and environmental factors in a sex-specific manner (Van Dongen et al., 2016).

Serotonin (5-HT) is a neurotransmitter involved in the regulation of mood and behavior, among others, and is known to be altered in internalizing disorders, particularly in depression (Krishnan and Nestler, 2008). Moreover, the human serotonergic system displays striking sex differences in its homeostasis. Baseline 5-HT function has been hypothesized to be higher in women than in men based on findings of increased (i) whole blood 5-HT levels, and (ii) brain expression of 5-HT_{1A} receptors, in women when compared to men (Ortiz et al., 1988; Parsey et al., 2002).

The serotonin transporter (SERT or 5-HTT) is one of the key elements involved in the complex regulation of serotonergic pathways. In this regard, the most prescribed antidepressants worldwide are selective serotonin reuptake inhibitors (SSRIs) (Olfson and Marcus, 2009); additionally, there is some evidence for the efficacy of this type of pharmacological treatment in somatization disorders, such as chronic functional pain conditions and fibromyalgia (Patetsos and Horjales-Araujo, 2016). As their name indicate, SSRIs act by pharmacologically

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blocking SERT, which is accompanied by a marked increase of 5-HT in the synaptic cleft. Interestingly, SSRIs are also used to treat other psychiatric disorders such as anxiety and obsessive compulsive disorder.

SLC6A4 gene encodes the serotonin transporter and has been thus extensively analyzed at a genetic and epigenetic level with regard to several complex disorders, with special emphasis on depression (Arias et al., 2005; Palma-Gudiel and Fañanás, 2017). A long polymorphic region in its promoter region, 5-HTTLPR, has received particular attention due to its functional relevance: 5-HTTLPR short allele (*s*) has been associated with decreased SERT expression when compared to its long allele (*l*). Nevertheless, genetic variability at this locus does not seem to robustly explain either vulnerability or course of illness in depressive disorders across studies (de Vries et al., 2016). Hence, attention is being paid to epigenetic signatures at the *SLC6A4* promoter region as a putative causal mechanism in the development of psychopathology.

Epigenetic mechanisms refer to chemical modifications found directly in the DNA sequence itself or in its packaging proteins, histones. Specifically, DNA methylation consists in the addition of a methyl group onto a cytosine residue belonging to a CpG dinucleotide. Although individual CpG sites can be found throughout the whole genome, CpG sites tend to cluster in specific regions of the genome called CpG islands. CpG islands are usually found in the promoter regions of genes and are characterized by extensive hypomethylation (that is, lack of DNA methylation). Interestingly, CpG islands' hypermethylation has been associated with decreased gene expression. In this regard, the *SLC6A4* gene contains a CpG island in its promoter region.

SLC6A4's CpG island methylation has been found to be associated with early or recent exposure to psychosocial stress together with a number of psychiatric disorders following a transdiagnostic pattern (Palma-Gudiel and Fañanás, 2017); however causality of such associations remains elusive due to the lack of longitudinal approaches. Interestingly, *SLC6A4* methylation was described to be positively associated with the presence of family history of depression rather than with treatment response suggesting epigenetic patterns may be heritable (Kang et al., 2013).

The aim of the current study was to investigate the role of *SLC6A4* methylation in anxious-depressive disorders and six dimensions of psychopathology in a sample of monozygotic twin pairs from the general population. To analyze this relationship, *SLC6A4* methylation was assessed by means of CpG probes included in a genome-wide array and further replicated by means of pyrosequencing technology. We hypothesized that (i) anxious-depressed subjects would exhibit higher *SLC6A4* methylation levels when compared to otherwise healthy subjects, (ii) *SLC6A4* methylation would be differentially correlated to internalizing and externalizing dimensions of psychopathology, (iii) sex would be a moderator factor in all associations, and (iv) *SLC6A4* methylation patterns would be highly correlated across co-twins.

2. Methods

2.1. Sample selection

Participants in this study were part of a larger sample recruited at the University of Barcelona (UB Twin Register). This sample consists of 230 adult Spanish dizygotic and monozygotic twins pertaining to the general population. Written informed consent, as approved by the local Ethics Committee, was obtained from all participants before assessment. Peripheral blood was obtained from all participants. A detailed description of sampling and methods can be found elsewhere (Córdova-Palomera et al., 2015). Eligibility criteria for the current epigenetic approach included monozygotic twin pairs for which blood samples and psychometric data were available for both twins of each pair. One hundred forty-eight subjects were eligible for the current epigenetic approach (Table 1, Replication sample).

Table 1
Summary and description of samples included.

	Testing sample	Replication sample	Brain sample
n	34	148[a]	39[b]
Tissue	Peripheral blood	Peripheral blood	Postmortem brain
DNA methylation assessment	Illumina 450 K BeadChip	Bisulfite pyrosequencing	Illumina 450 K Beadchip
Mean age (range)	35.5 (19, 54)	35.5 (17, 68)	73 (15, 114)
Sex (F:M)	16:18	90:58	19:20

[a] One hundred forty-eight subjects included in the Replication sample comprise the thirty-four subjects of the testing sample.
[b] Actual number of samples assayed in the 450 K array in the Brain sample amounts to 260 since several samples from different brain sources were included for each subject.

2.2. Clinical evaluation

Lifetime psychiatric history was screened by trained psychologists following the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First et al., 1996). Because of both the relatively low prevalence rates of individual psychiatric disorders in the general population and the higher prevalence of anxiety and depressive disorders relative to other disorders, these diagnoses were subsumed under a single diagnostic category of “anxious-depressive disorders”. This classification has been used in previous studies conducted in the UB Twin Register sample revealing common biological pathways for such disorders (Córdova-Palomera et al., 2015). This category included the DSM-IV categorical diagnoses of: major depressive disorder (either single episode, recurrent or non-otherwise specified), anxiety disorder (with or without agoraphobia, and non-otherwise specified) and phobias (either specific or social). Specifically, in our sample 40.0% of women (n = 36) and 12.1.8% of men (n = 7) had a lifetime diagnostic of any anxious-depressive disorder; additionally, three subjects met DSM-IV criteria for anorexia nervosa, bulimia and psychotic disorder non-otherwise specified, respectively. Out of 46 subjects affected by any psychiatric disorder, 19 subjects exhibited DSM-IV Axis I comorbidity. Twenty-five subjects (54% of the affected ones) met DSM-IV Axis I criteria for current psychiatric disorders.

Additionally, on the same day of blood sampling, current psychiatric symptoms were evaluated using the Brief Symptom Inventory (BSI). The BSI is a self-administered scale consisting of 46 items to quantify the experience of psychopathological symptoms of a broad nature during the last 30 days including six subscales: depression, phobic anxiety, somatization, obsession-compulsion, hostility and paranoid ideation (Derogatis and Spencer, 1982). The BSI has been validated as a reliable tool for the assessment of symptom-based psychopathological dimensions in non-clinical samples (Ruipérez et al., 2001). Further details on specific BSI scores in the currently assessed sample can be found in Table 2.

Table 2
Age at assessment and BSI dimensions scores of assessed subjects (from the Replication sample) according to sex.

Mean age	Women (n = 90)	Men (n = 58)	p-Value
BSI Dimensions			
Mean age at assessment (SD)	34.4 (13.6)	37.3 (13.3)	0.2
BSI total score	22.8 (21.1)	14.3 (12.1)	0.002
BSI depression score	5.4 (5.4)	3.0 (2.9)	0.001
BSI anxiety score	1.8 (3.1)	0.8 (1.3)	0.006
BSI obsessive compulsive score	6.0 (5.5)	4.1 (4.1)	0.02
BSI somatization score	4.0 (4.2)	2.3 (2.6)	0.003
BSI hostility score	1.0 (2.0)	0.9 (1.2)	0.6
BSI paranoid score	4.8 (4.4)	3.4 (2.9)	0.02