Available online at www.sciencedirect.com
ScienceDirect
Journal of Psychiatric Research 42 (2008) 50–57
JOURNAL OF PSYCHIATRIC RESEARCH
www.elsevier.com/locate/jpsychires

Contribution of the serotoninergic system to anxious and depressive traits that may be partially responsible for the phenotypical variability of bulimia nervosa

Marta Ribasés [a,b], Fernando Fernández-Aranda [c], Mònica Gratacòs [a,d], Josep M. Mercader [a], Carolina Casasnovas [c], Araceli Núñez [c], Julio Vallejo [c], Xavier Estivill [a,d,e,*]

[a] *Genes and Disease Program, Center for Genomic Regulation, Barcelona Biomedical Research Park, Barcelona, Catalonia, Spain*
[b] *Department of Psychiatry, Hospital Universitari Vall d'Hebron, Barcelona, Catalonia, Spain*
[c] *Department of Psychiatry, University Hospital of Bellvitge, L'Hospitalet de Llobregat, Barcelona, Catalonia, Spain*
[d] *National Genotyping Center (CeGen), Barcelona Node, Center for Genomic Regulation, Barcelona Biomedical Research Park, Barcelona, Catalonia, Spain*
[e] *Experimental and Health Sciences Department, Pompeu Fabra University, Barcelona Biomedical Research Park, Barcelona, Catalonia, Spain*

Received 24 May 2006; received in revised form 31 July 2006; accepted 1 September 2006

Abstract

Eating disorders (ED), such as anorexia nervosa (AN) and bulimia nervosa (BN), are complex psychiatric phenotypes influenced by both genetic and environmental factors. We investigated the genetic contribution of four single nucleotide polymorphisms (SNPs) within the serotonin receptor *5HT2C* and two sequence variants within the serotonin transporter *SLC6A4* to different ED-related psychopathological symptoms in a total sample of 82 ED patients. All patients were diagnosed according to DSM-IV criteria and underwent diagnostic and psychopathological assessments by means of structured clinical interviews and rating scales. We detected significant evidence of association between the −995A/−759T/−697C/Cys23 haplotype of the *5HT2C* gene and different anxious and depressive subscales of the SCL90-R instrument, that included Somatization ($p = 0.029$), Obsessive-Compulsiveness ($p = 0.021$), Depression ($p = 0.032$), Anxiety ($p = 0.004$), Hostility ($p = 0.028$), Phobic Anxiety ($p = 0.029$) and Paranoid Ideation ($p = 0.008$), in BN patients. We also observed a strong association between the 5HTTLPR polymorphism of the *SLC6A4* gene and Anxiety in the same group of BN patients ($p = 0.004$). However, no epistatic effects between the *5HT2C* and *SLC6A4* genes on the different anxious and depressive subscales were observed. Our preliminary data suggest that the serotoninergic system contributes to the different psychopathological symptoms that may be partially responsible for the phenotypical variability within the bulimic phenotype.

Keywords: Eating disorders; Bulimia nervosa; Serotonin; Psychopathological symptoms; *5HT2C*; *SLC6A4*

1. Introduction

Eating disorders (ED), such as anorexia nervosa (AN) and bulimia nervosa (BN), are complex psychiatric phenotypes influenced by both genetic and environmental factors (Fairburn and Harrison, 2003). It has been reported a high comorbidity between ED and Axis I or Axis II disorders, such as affective disorders, personality disorders, anxiety disorders, impulse control disorders and substances abuse (Bulik et al., 2004; Fernández-Aranda et al., in press-a; Godart et al., 2000; Grilo et al., 2003; Kaye et al., 2004; Milos et al., 2004; Solano et al., 2005; Steiger et al., 2005). Different lines of investigation show that certain ED-related personality and psychopathological traits persist after normalization of body weight. These traits are known to be heritable and may be partially responsible

* Corresponding author. Address: Genes and Disease Program, Center for Genomic Regulation, Barcelona Biomedical Research Park, Passeig Maritim, 37-49, 08003 Barcelona, Catalonia, Spain. Tel.: +34 93 224 0959; fax: +34 93 224 0899.
E-mail address: xavier.estivill@crg.es (X. Estivill).

0022-3956/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jpsychires.2006.09.001

for the genetically driven phenotypical variability within the different ED categories and could influence susceptibility to AN and BN (Augestad et al., 1999; Bean et al., 2005; Blonigen et al., 2005; Cassin and von Ranson, 2005; Fassino et al., 2002; Lilenfeld et al., 2005; Reba et al., 2005). Genes involved in the serotoninergic system, such as the serotonin receptor *5HT2C* and the serotonin transporter *SLC6A4*, are good candidates for their involvement in some of the phenotypical traits postulated to underlie ED, such as anxiety, hostility, depression, aggressiveness and impulsivity (Fernández-Aranda et al., in press-b; Frankle et al., 2005; Lesch et al., 1996; Nonogaki et al., 1998; Tsai et al., 2002; Willis-Owen et al., 2005).

The *5HT2C* gene maps to human chromosome Xq24, is widely expressed throughout the central nervous system, including the hypothalamus nuclei that control body weight, and its disruption causes obesity and hyperphagia in the *5ht2c* (−/−) knock-out mice (Milatovich et al., 1992; Nonogaki et al., 1998; Tecott et al., 1995). Together with the *5HT2C* receptor, the serotonin transporter 5HTT, which is encoded by a single gene (*SLC6A4*) on human chromosome 17q12, is essential for the serotoninergic activity by regulating the magnitude and duration of serotoninergic responses (Blakely et al., 1991).

Different studies have shown a strong association between *5HT2C* and *SLC6A4* and different anxiety-related personality traits using different inventories (Ebstein et al., 1997; Kuhn et al., 1999; Lesch et al., 1996; Melke et al., 2001; Munafo et al., 2005). Based on these investigations we hypothesized that psychopathological traits associated to ED are substantially influenced by alterations in the serotonin pathways. To test this hypothesis, we have examined the involvement of the *5HT2C* and *SLC6A4* genes in the psychopathological symptomatology measured by the SCL90-revised questionnaire (SCL90-R) in a total sample of 82 patients with ED.

2. Materials and methods

2.1. Subjects

The clinical group consisted of 82 unrelated Caucasian ED patients of Spanish origin consecutively referred for assessment and treatment at the Department of Psychiatry of the Bellvitge University Hospital in Barcelona, between 1999 and 2002. All individuals were diagnosed according to DSM-IV criteria (American Psychiatric Association, 1994) using a semi-structured clinical interview with the Structured Clinical Interview for Mental disorders v.2.0 (SCID-I) and completed the Symptom Checklist-90-revised inventory (SCL90-R). The studied sample consisted of 36 BN patients (43.9%), all of them of purging subtype, and 46 AN patients (56.1%; 19 restricting versus 27 binge-eating/purging AN). More than 3 years of restrictive illness were necessary to classify patients as ANR. All ED patients were female and clinical information was available from most of the patients. The average age at assessment was 24.6 years (SD = 4.4) for BN patients and 25.1 years (SD = 6.5) for AN patients. The lifetime minimum body mass index (minBMI) was 18.9 kg/m^2 (SD = 2.7) for BN patients and 15.4 kg/m^2 (SD = 1.4) for AN patients. The average age at onset of the disorder was 17.1 years (SD = 3.3) for BN patients and 18.6 years (SD = 4.6) for AN patients. Diagnosis was blind to genotype and most of the patients have been described in previous reports (Gabrovsek et al., 2004; Ribases et al., 2003; Ribases et al., 2005a; Ribases et al., 2004; Ribases et al., 2005b). The study was approved by the ethic's committee of the Institution, and written informed consent was obtained from all subjects.

2.2. Clinical assessment

Demographic-clinical information including age, weight, height and clinical-psychopathological variables were also obtained. Additional demographic information including education, occupation and living arrangements was obtained via semi-structured interview. The patients completed the SCL-90-R, that is a widely used 90-item scale for assessing self-reported psychological distress and psychopathology (Derogatis, 1990; Derogatis and Melisaratos, 1983). The SCL90-R inventory measures a broad range of psychological problems and symptoms of psychopathology through three Global Indexes (Global Severity Index, Positive Symptom Distress Index and Positive Symptom Total) and nine primary symptom dimensions comprising a total of 83 items (Somatization, Obsessive-Compulsive, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic Anxiety, Paranoid Ideation and Psychoticism). The Global Severity Index, which is the participant's mean score (using all 90 items), is a widely used global index of distress. This scale has been validated in Spanish population, obtaining a mean internal consistency of 0.75 (Coefficient alpha; Derogatis, 2002).

2.3. Genotyping

The *5HT2C* SNPs were genotyped from each DNA sample by the polymerase chain reaction (PCR) in a total volume of 10 μl, containing 50 ng of template DNA, 50 mM KCl, 10 mM Tris–HCl, 1.5 mM $MgCl_2$, 200 μM of dNTPs, 10 pmol of each oligonucleotide and 0.25 U of *Taq* DNA polymerase. Amplification conditions consisted of an initial 4 min denaturation step at 94 °C, 35 cycles of 30 s at 94 °C, 45 s at 58 °C and 45 s at 72 °C, followed by a final extension of 10 min at 74 °C. To genotype the −995G > A (rs3813928) variant amplification was performed with primers 5′-CTTGAAGGGAGTTTCAAAGC-3′ and 5′-CCGGTCTCTTAGTGCATCTG-3′ and PCR products were digested with *Rsa*I. Digestion with *Rsa*I yields three fragments (168, 71 and 68 bp) in the case of −995G and two (239 and 68 bp) in the case of -995A. Genotyping of the −759C > T (rs3813929) and −697G > C (rs518147) was with primers 5′-ATCTCCACCATGGGTCTCGC-3′